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

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Summary

- Leaf aging 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 1099 leaves from 12 lowland Amazonian canopy trees in southern Peru.
- Results demonstrated monotonic decreases in leaf water (LWC) and phosphorus (Pmax) contents and an increase in leaf mass per unit area (LMA) with age across trees; leaf nitrogen (Nmax) and carbon (Cmax) contents 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 ($R^2 = 0.86$; percent root mean square error (%RMSE = 33) compared with trait-based models using single ($R^2 = 0.07-0.73$; %RMSE = 7–38) and multiple ($R^2 = 0.76$; %RMSE = 28) predictors. Spectra- and trait-based models established a physiochemical basis for the spectral age model. Vegetation indices (VIs) including the normalized difference vegetation index (NDVI), enhanced vegetation index 2 (EVI2), normalized 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.

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., 2000, 2004; Saleska et al., 2003, 2007; Huete et al., 2006; Myneni et al., 2007; Doughty & Goulden, 2008; Anderson et al., 2010, 2011; Brando et al., 2010; Samanta et al., 2010, 2012; Bradley et al., 2011; Anderson, 2012; Morton et al., 2014) as they contain 55% of the global carbon stores (Pan et al., 2011) and account for c. 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 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-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 ‘optimized’ index designed to not saturate in high-biomass regions such as the tropics; Huete et al., 2006) 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., 1998; Miller et al., 1991; Reich et al., 1991; Kitajima et al., 1997, 2002; Escudero & Mediavilla, 2003; Mediavilla et al., 2011), have been shown to directly influence the spectral reflectance behaviour of leaves (Gausman et al., 1970; Knipling, 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 (Pmass), nitrogen (Nmass) and carbon (Cmass) contents) changes associated with natural leaf aging 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 aging? (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 aging 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 C. Chavana-Bryant et al. (unpublished) we explore the role of leaf age as a fundamental driver of intraspecific trait variation within Amazonian canopy trees. In a follow-up paper, J. Wu et al. (unpublished) 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.

**Materials and Methods**

**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., 2002) 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 4–5-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 because of severe droughts in 2005 and 2010; 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 brevideciduous 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 brevideciduous phenology (i.e. initiated leaf flushing when most senescent leaves had been abscised or after 1–4 wk 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 c. 50% of its canopy through a prolonged period of leaf abscission and flushing that lasted the entire dry season.

**Tree sampling strategy**

The tree sampling for this study focused on canopy and emergent trees (Table 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 171 cm (range 117–237 cm). The sampled trees averaged 32 m in height (range 29–35 m), compared with a mean (±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 9.6 m (range 6.3–15.1 m).
We sampled the developmental, mature and senescent leaf phases (the detailed leaf age classification used in this study can be found in Fig. 1) for eight trees and also sampled some leaf ages from four other trees (see Supporting Information Table S1 and the ‘Branch sampling’ section in Methods S1). 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 wk 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).

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 waxinesses. This sampling allowed us to examine the variability attributable to natural leaf aging of a range of leaf types, sizes, shapes, thicknesses and waxinesses 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.

Leaf age classification

We selected LMA, LWC, P\textsubscript{mass}, N\textsubscript{mass} and C\textsubscript{mass} as the ensemble of leaf traits to measure (full methodological details are included in Methods S1). 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 C. Chavana-Bryant et al. (unpublished). We assessed leaf waxiness qualitatively according to leaf glossiness and categorized the eight main tree species into S. amara, Licania brittoniana and Clarisia racemosa as having high leaf waxiness, and Ruizodendron ovale, Couratari macroperma, Bertholletia excelsa, Guatteria boliviana and Pouteria franciscana as having low leaf waxiness.

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 350–2500 nm and sampling intervals of 1.4 nm from 350 to 1000 and 2 nm from 1000 to 2500 nm. The spectrometer was fitted with a contact probe (High Intensity
Leaf developmental phase ending in maturity

<table>
<thead>
<tr>
<th>Age codes</th>
<th>Age (wk)</th>
<th>Leaf description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y1</td>
<td>1</td>
<td>Young1 – recently emerged leaf, sampled after 1 wk of active leaf expansion. Leaf very fragile and thin, small in size, has no rigidity (will not fold when held up), its colour may be yellow or light green.</td>
</tr>
<tr>
<td>Y2</td>
<td>2</td>
<td>Young2 – leaf sampled after 2 wk of active leaf expansion. Leaf still fragile and thin, has not achieved full size, has some rigidity (will not fold if held up), its colour is definitely light green.</td>
</tr>
<tr>
<td>Y3</td>
<td>3</td>
<td>Young3 – leaf sampled after 3 wk of active leaf expansion. Leaf not fragile any more, but still thin or close to achieving full thickness and rigidity, has almost achieved full size or achieved full size, its colour is darker green.</td>
</tr>
<tr>
<td>M</td>
<td>7–35</td>
<td>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. Individual trees sampled for up to 3 months for this age class.</td>
</tr>
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Leaf senescent phase ending in abscission

<table>
<thead>
<tr>
<th>Age codes</th>
<th>Age (wk)</th>
<th>Leaf description</th>
</tr>
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<tbody>
<tr>
<td>O</td>
<td>36–44</td>
<td>Old – leaf near the end of its life cycle (c. 2 months before senescence); distinguished from previous age class by darker (shade leaf) and/or yellower (sun leaf) colouration, and by initial signs of epiphylls and/or senescence. Individual trees sampled for up to two consecutive months.</td>
</tr>
<tr>
<td>S</td>
<td>45–57</td>
<td>Senescent – leaf in the process of dying and abscising; distinguished by loss of chlorophyll and/or onset of decomposition. Individual trees sampled for up to three consecutive months for this age class.</td>
</tr>
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</table>

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, which is part of a doctoral research project, can be seen in https://vimeo.com/152464003.

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 (Fig. 2). For the
same subset of trees, we calculated and compared mean reflectance (%; Fig. 3) and reflectance variability (measured as coefficients of variation (CVs); bottom graph in Figs 4 and S1) 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. In order 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\textsubscript{mass}, N\textsubscript{mass} and C\textsubscript{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.

PLSR is a standard statistical approach utilized in chemometric analyses and is designed to handle high predictor collinearity and situations where the number of predictor variables (i.e. 2151 wavelengths) is much higher than the number of observations. PLSR reduces the large predictor matrix down to a relatively few, uncorrelated latent factors. As in Asner et al. (2011a,b, 2014, 2015) and Serbin et al. (2014), we performed a one-time randomized 70 : 30 split of our data into calibration and independent testing sets. We adopted a 100× permutation 10-fold cross-validation (i.e. 100 total permutations using 90% of the calibration data set with 10% used as 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 minimized RMSE and maximized \( R^2 \).

The model performance was further characterized 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,b) (Table 2).

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 (Figs 5, S2), and examined the variation explained by the PLSR latent factors for leaf age and traits (Fig. 6). 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).

Finally, we explored the effect that changes in leaf reflectivity attributable to age can have on VIs commonly used to measure vegetation greenness (normalized difference vegetation index (NDVI) and enhanced vegetation index (EVI)), light-use efficiency (photosynthetic reflectance index (PRI)) and water content (normalized 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 (NIR) as a result of 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 summarized in Table S2. 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) (Fig. 7). We report differences (measured in % change) in VIs attributable to age.

**Results**

**Age-dependent morphological and biochemical leaf properties**

LWC, LMA and \( P_{\text{mass}} \) 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 \( P_{\text{mass}} \), \( R^2 = 0.60–0.93 \) in...
individuals and \( R^2 = 0.44 \) across the community; all \( P \)-values < 0.0001 (Fig. 2; see also Fig. S3). \( N_{\text{mass}} \) 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). \( C_{\text{mass}} \) 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 C. Chavana-Bryant et al. (unpublished).

In addition to varying age–trait relationships across different leaf traits, we found that relationships also varied across individual trees for both \( N_{\text{mass}} \) and \( C_{\text{mass}} \). \( N_{\text{mass}} \) increased or decreased either logarithmically or linearly across trees, while \( C_{\text{mass}} \) either remained stable or increased linearly or decreased logarithmically with age. LWC, LMA and \( P_{\text{mass}} \) showed similar age–trait relationships among individual trees, with LWC and \( P_{\text{mass}} \) displaying a logarithmic decrease and LMA a logarithmic increase.

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 (Fig. 3). The main observed patterns of age-related spectral changes were: visible (VIS; spectral domain: 390–700 nm) 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 550 nm (green peak); NIR reflectance (spectral domain: 700–1400 nm) 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 700–850 nm; shortwave-infrared (SWIR; spectral domain: 1450–2500 nm) continuously increased as leaves aged in low-wax leaves, while high-wax leaves displayed less change, with structural carbon absorption features appearing at c. 2000–2250 nm during senescence; and red-edge (spectral domain: 650–810 nm) 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 Fig. S1.
We also observed age effects in terms of reflectance variability (measured as CV) across individual trees (bottom graph in Figs 4, S4). 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 850 nm, which showed variability (CV = 4–35%) with a higher water absorption feature at c. 1450 and 1940 nm showing peaks in variability (CV = 4–18% and 7–40%, respectively). Furthermore, variability was higher in the SWIR 2 region (1940–2450 nm) 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 (Fig. S4).

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 (Table 2) 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 wk. 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.

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 (Fig. S5), 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, Nmass and Cmass, 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 3). Model 2 (LWC excluded) included Pmass LMA and Nmass, 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 3).

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 Pmass and LMA, the next most important traits, had contributions of $R^2 = 0.45$ and $R^2 = 0.05$, respectively. Both Nmass and Cmass had negligible contributions (see Table 3). 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 Fig. 2), where we observed that LWC displayed the strongest trait–age relationship, followed by Pmass and LMA. Furthermore, due to negative correlation between LMA and LWC (see Fig. S5), most of the contribution of LMA to leaf age prediction is included within the LWC contribution to the model.

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 (Figs 5, S2) revealed that the strongest relationship was found between leaf age and LWC weights across all five factors ($R^2 = 0.53–0.91$; all $P$-values < 0.0001), followed by LMA ($R^2 = 0.12–0.95$; all $P$-values < 0.0001) and Pmass ($R^2 = 0.04–0.81$; all $P$-values < 0.0001). Factor weights for both Nmass ($R^2 = 0.03–0.56$; all $P$-values < 0.0001) and Cmass ($R^2 = 0.16–0.46$; 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 (Fig. 6). The patterns of variation explained by each latent factor suggest close spectral relationships between leaf age, LWC, LMA and Pmass, while the patterns of variation explained for both Nmass and Cmass indicated that they might provide different, but complementary, information to the overall spectral age relationship.

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) (Fig. 7). 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), 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.

Fig. 7 Variation in vegetation indices (VIs) commonly used to measure vegetation greenness (broadband normalized 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.

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 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 S2) 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 Fig. 4). The red edge VIs capture this spectral domain, and thus are the most sensitive to leaf age.

**Discussion**

To our knowledge, our study is the first to comprehensively analyse the morphological (LMA), biochemical (LWC, Pmass, Nmass and Cmass) 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 waxinesses. This, together with the variation of these leaf traits across the leaf lifecycle (see Table 4), 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%), P (0.04%), N (0.85%) and C (36%) – 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. (2011a,b) 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, 2002) and nontropical forests (Field & Mooney, 1983; Escudero & Mediavilla, 2003; Niinemets & Kull, 2003; Niinemets et al., 2004, 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 Pmass display age-dependent monotonic increases (LMA) or decreases (LWC and Pmass) across individual trees, with the variation of these leaf traits across the leaf lifecycle (see Table 4). Across all trees, reflectance in the visible spectrum (VIS; 412–700 nm) which is dominated by pigment (chlorophylls, anthocyanins and carotenoids) light absorption (Knapling, 1970; Elvidge, 1990) and controlled by the amount and status of these pigments (Billings & Morris, 1951), showed a concave response with age (Fig. 3). Reflectance decreased continuously across this spectral domain as chlorophyll developed in young leaves, with the most marked changes observed at the green peak (550 nm); following maturity, reflectance then increased throughout the VIS but with marked increases at c. 570–630 nm 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), Boocbs et al. (1990) and Roberts et al. (1998), we observed red edge shifts attributable to leaf age. NIR reflectance (700–1400 nm) 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 et al., 1970; Woolley, 1971). However, epi-cuticular 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

Table 3 Results of step-wise (forward) regression modelling for leaf age (log-transformed) using leaf water content (LWC), phosphorus content (Pmass), nitrogen content (Nmass) and carbon content (Cmass) as predictor parameters for Model 1 and leaf mass per unit area (LMA), Pmass, Nmass and Cmass for Model 2

<table>
<thead>
<tr>
<th>Model 1 summary</th>
<th>Model step</th>
<th>Selected parameters</th>
<th>R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>R² = 0.76</td>
<td>1</td>
<td>LWC</td>
<td>0.7339</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>RMSE = 2.68</td>
<td>2</td>
<td>Nmass</td>
<td>0.7487</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>%RMSE = 32</td>
<td>3</td>
<td>Cmass</td>
<td>0.7584</td>
<td>0.0003</td>
</tr>
<tr>
<td>AICc = 735.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2 summary</th>
<th>Model step</th>
<th>Selected parameters</th>
<th>R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>R² = 0.50</td>
<td>1</td>
<td>Pmass</td>
<td>0.4484</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>RMSE = 3.81</td>
<td>2</td>
<td>LMA</td>
<td>0.5019</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>%RMSE = 46</td>
<td>3</td>
<td>Nmass</td>
<td>0.5042</td>
<td>0.2269</td>
</tr>
<tr>
<td>AICc = 939.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Root mean square error (RMSE) and %RMSE are reported on the original scale. AIC, Akaike information criterion.
marked collapse in NIR reflectance between 700 and 850 nm due to decreased photon scattering as a result of the breakdown of intercellular structure as leaves senesced. The shortwave infrared spectral domain (SWIR: 1400–2500: 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 senescence.

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.

**Spectral leaf age model: underlying mechanisms**

The results of our PLSR modelling demonstrate the ability of spectroscopic data to characterize leaf age for individual trees ($R^2 = 0.91–0.98$; %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 2). We found that trees with leaves of different spectral properties aged in a similar manner (Fig. 3) 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 (Fig. 2) or as multiple predictors in a step-wise regression model (Table 3) to predict leaf age, our PLSR approach captures the simultaneous contribution of many important absorption properties of leaves (Fig. 4) 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) (Fig. 4). 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

**Table 4**: 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 class)

<table>
<thead>
<tr>
<th>Leaf age class</th>
<th>Leaf traits</th>
<th>LMA (g m$^{-2}$)</th>
<th>LWC (%)</th>
<th>$P_{\text{mass}}$ (%)</th>
<th>$N_{\text{mass}}$ (%)</th>
<th>$C_{\text{mass}}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y1 M (SD)</td>
<td></td>
<td>72.8 (25.1)</td>
<td>71 (5)</td>
<td>0.23 (0.08)</td>
<td>2.66 (1.03)</td>
<td>50.22 (2.29)</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>34.6–135.5</td>
<td>60–81</td>
<td>0.11–0.40</td>
<td>0.85–4.19</td>
<td>46.74–55.13</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>121</td>
<td>121</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Y2 M (SD)</td>
<td></td>
<td>85.4 (34.9)</td>
<td>68 (6)</td>
<td>0.22 (0.06)</td>
<td>2.77 (0.82)</td>
<td>49.66 (2.16)</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>37.6–187.4</td>
<td>55–79</td>
<td>0.10–0.31</td>
<td>1.46–4.08</td>
<td>45.46–53.60</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>110</td>
<td>110</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Y3 M (SD)</td>
<td></td>
<td>96.9 (26.4)</td>
<td>63 (5)</td>
<td>0.18 (0.06)</td>
<td>2.55 (0.69)</td>
<td>49.12 (1.60)</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>47.4–166.1</td>
<td>51–73</td>
<td>0.08–0.33</td>
<td>1.45–3.69</td>
<td>45.28–52.87</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>110</td>
<td>110</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>M M (SD)</td>
<td></td>
<td>122.5 (26.3)</td>
<td>53 (5)</td>
<td>0.11 (0.03)</td>
<td>2.19 (0.54)</td>
<td>50.33 (3.95)</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>76.1–199.7</td>
<td>44–62</td>
<td>0.06–1.19</td>
<td>1.40–3.68</td>
<td>45.04–59.72</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>230</td>
<td>230</td>
<td>65</td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td>O M (SD)</td>
<td></td>
<td>138.7 (28.3)</td>
<td>48 (5)</td>
<td>0.11 (0.04)</td>
<td>2.14 (0.34)</td>
<td>51.73 (4.58)</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>90.4–202.7</td>
<td>38–60</td>
<td>0.05–0.18</td>
<td>1.42–2.93</td>
<td>38.92–59.47</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>150</td>
<td>150</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>S M (SD)</td>
<td></td>
<td>137.0 (32.2)</td>
<td>48 (5)</td>
<td>0.11 (0.04)</td>
<td>2.16 (0.47)</td>
<td>50.65 (4.51)</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>79.4–210.5</td>
<td>34–58</td>
<td>0.04–0.18</td>
<td>1.07–2.89</td>
<td>35.83–57.39</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>321</td>
<td>321</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>All M (SD)</td>
<td></td>
<td>116.45 (37.73)</td>
<td>56 (10)</td>
<td>0.15 (0.07)</td>
<td>2.37 (0.68)</td>
<td>50.34 (3.72)</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>34.60–210.5</td>
<td>34–81</td>
<td>0.04–0.40</td>
<td>0.85–4.19</td>
<td>35.83–59.72</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>1099</td>
<td>1099</td>
<td>315</td>
<td>315</td>
<td>315</td>
</tr>
</tbody>
</table>

M, mean; range, minimum and maximum values; n, number of samples. Leaf age classes: Y1, Y2 and Y3 are developing leaves collected after 1, 2 and 3 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.

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descending order of importance) from LMA, $P_{\text{mass}}$, N$_{\text{mass}}$ and $C_{\text{mass}}$.

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 1940 nm). 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 700–850 nm), 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), Asamaa et al. (2005) and Castro & Sanchez-Azofeifa (2008)). The relationship between leaf age and $P_{\text{mass}}$ and N$_{\text{mass}}$ 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 550 nm), RE (680–750 nm) and SWIR (1510, 1690, 1940 and 2240 nm). The spectral leaf age and $C_{\text{mass}}$ relationship is reflected in PLSR coefficient peaks at 1420–1450, 1490, 1690, 1820, 1940 and 2340 nm and related to structural carbohydrates: polyphenolics and cellulose.

Similar patterns of age-related variation in leaf reflectivity (Figs 3, 4) to those we discuss in the ‘Variation of leaf spectral characteristics with age and their relationship to leaf structure and biochemistry’ subsection have also been reported for non-tropical 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 waxes and LWC/LMA.

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 (Fig. 7). 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 (Fig. 4). 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, 2008b). 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. (2006) across seasonally dry Amazon forests. Furthermore, field-based studies (Doughty & Goulden, 2008; Chave et al., 2009; Brando 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. (2006), 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.

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 (J. Wu et al., unpublished) provide initial 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.

Acknowledgements

This research was supported by an NERC TROBIT project (NE/D005469/1) student grant to C.C-B. with additional grant support to F.F.G. from the Centre for Ecology & Hydrology (CEH). Y.M. is supported by the Jackson Foundation and an ERC Advanced Investigator Grant GEM-TRAIT (321131). We thank Olivier Jaudoin, Michael Eltringham, Stefan Curtis, Ana Lombardero Morán, Valentine Alt and Italo Treviño Zeballos for excellent field assistance. We also thank PUCP staff (Fabian Limonchi Tamamoto and Eliana Esparza Ballón) for vital support with permits and logistics in Peru, NERC NSF (Alasdair MacArthur and Christopher Macellon) and CEH staff (Charles George, Cyril Barrett, Dave McNeil, Alan Warwick and Geoff Wicks) for invaluable field equipment support, University of Arizona technicians (Vanessa Buzzard and Chris Eastoe) for leaf chemical processing, Dan Metcalfe for assisting with leaf area calculations, SERNANP for granting research/collection permissions and Explorers’ Inn Tambopata for accommodation. The authors also thank the anonymous reviewers who contributed to the improvement of this manuscript.

Author contributions

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.

References


Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Difference in mean reflectance between mature leaves and other leaf age classes for sun leaves of eight Amazonian canopy and emergent trees.

Fig. S2 Spectral weights (w*) for leaf age, leaf mass per unit area (LMA), leaf water content (LWC), phosphorus content (Pmass), nitrogen content (Nmass) and carbon content (Cmass).

Fig. S3 Relationships between leaf traits and leaf age (not including developing leaves).

Fig. S4 Spectral coefficients of reflectance variation (CV) by leaf age class for sun leaves of eight Amazonian canopy and emergent trees.

Fig. S5 Relationship between leaf water content (LWC) and leaf mass per unit area (LMA).

Table S1 Leaf sampling scheme by tree, canopy position (SU = sun) and leaf age class.

Table S2 List of vegetation indices (VIs) and corresponding equations used in this study.

Methods S1 Branch sampling and leaf traits and measurements.

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