Convergence in relationships between leaf traits, spectra and age across diverse canopy environments and two contrasting tropical forests

Jin Wu[1,10], Cecilia Chavana-Bryant[2], Neill Prohaska[1], Shawn P. Serbin[3], Kaiyu Guan[4], Loren P. Albert[1], Xi Yang[5], Willem J. D. van Leeuwen[6], Anthony John Garnello[1], Giordane Martins[7], Yadvinder Malhi[2], France Gerard[8], Raimundo Cosme Oliviera[9] and Scott R. Saleska[1]

[1]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, USA; [2]Environmental Change Institute, School of Geography and the Environment, University of Oxford, Oxford, OX1 3QY, UK; [3]Biological, Environmental & Climate Sciences Department, Brookhaven National Lab, Upton, New York, NY 11973, USA; [4]Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana Champaign, Urbana, IL 61801, USA; [5]Department of Earth, Environmental, and Planetary Sciences, Brown University, Providence, RI 02912, USA; [6]School of Natural Resources and the Environment, University of Arizona, Tucson, AZ 85721, USA; [7]Brazil's National Institute for Amazon Research (INPA), Manaus, AM 69067-375, Brasil; [8]Centre for Ecology and Hydrology (CEH), Wallingford, OX10 8BB, UK; [9]Embrapa Amazônia Oriental, Santarém, PA 68035-110, Brasil; [10]Present address: Biological, Environmental & Climate Sciences Department, Brookhaven National Lab, Upton, New York, NY 11973, USA

Authors for correspondence:
Jin Wu
Tel: +1 520 704 5358
Email: jinwu@bnl.gov

Scott R. Saleska
Tel: +1 520 626 1500
Email: saleska@email.arizona.edu

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Summary

- Leaf age structures the phenology and development of plants, as well as the evolution of leaf traits over life histories. However, a general method for efficiently estimating leaf age across forests and canopy environments is lacking.
- Here, we explored the potential for a statistical model, previously developed for Peruvian sunlit leaves, to consistently predict leaf ages from leaf reflectance spectra across two contrasting forests in Peru and Brazil and across diverse canopy environments.
- The model performed well for independent Brazilian sunlit and shade canopy leaves ($R^2 = 0.75$–0.78), suggesting that canopy leaves (and their associated spectra) follow constrained developmental trajectories even in contrasting forests. The model did not perform as well for mid-canopy and understory leaves ($R^2 = 0.27$–0.29), because leaves in different environments have distinct traits and trait developmental trajectories. When we accounted for distinct environment–trait linkages – either by explicitly including traits and environments in the model, or, even better, by re-parameterizing the spectra-only model to implicitly capture distinct trait-trajectories in different environments – we achieved a more general model that well-predicted leaf age across forests and environments ($R^2 = 0.79$).
- Fundamental rules, linked to leaf environments, constrain the development of leaf traits and allow for general prediction of leaf age from spectra across species, sites and canopy environments.

Introduction

It has long been recognized that many important ecological processes vary with leaf age, the time elapsed since leaf budburst. During their lifetime, leaves exhibit variable photosynthetic rates (Field, 1983; Reich *et al.*, 1991; Wilson *et al.*, 2001; Kitajima *et al.*, 2002; Pantin *et al.*, 2012), morphological changes (Maksymowych, 1973), allocation and transformation of chemicals (Wilson *et al.*, 2001; Kitajima *et al.*, 2002; Pantin *et al.*, 2012), epiphyll colonization (Roberts *et al.*, 1998; Toomey *et al.*, 2009) and defense against herbivory (Coley, 1980; Coley & Barone, 1996; Lawrence *et al.*, 2003; Wang *et al.*, 2012). Thus, leaf age is a critical parameter for interpreting leaf function over time and for understanding how leaf traits evolve over development. Furthermore, expected maximum leaf age (leaf lifespan) is central to understanding plant life history (Field & Mooney, 1983; Reich *et al.*, 1992), population dynamics (Reich *et al.*, 2004) and the evolutionary trade-offs of the leaf economic spectrum (Reich *et al.*, 1997; Wright *et al.*, 2004; Funk & Cornwell, 2013; Osnas *et al.*, 2013). Thus, many disciplines have long been interested in monitoring leaf age for individual plants (Field, 1983; Roberts *et al.*, 1998; Wilson *et al.*, 2001; Reich *et al.*, 2004) and leaf lifespan for many species (Reich *et al.*, 1991, 1992; Wright *et al.*, 2004; Funk & Cornwell, 2013; Osnas *et al.*, 2013).

More recent studies have begun to emphasize the importance of leaf ages and canopy age composition on phenology and ecosystem seasonality of vegetation photosynthesis and transpiration (Doughty & Goulden, 2008; Richardson *et al.*, 2012; Restrepo-Coupe *et al.*, 2013; Wu *et al.*, 2016). Yet leaf development is difficult to monitor at large scales, especially in carbon-

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rich tropical evergreen forests, where individual leaf ages are not as tightly synchronized with phenology and ecosystem seasonality as in temperate forests (Reich, 1995). In tropical forests, contrasting interpretation of satellite-detected seasonality of vegetation greenness (Morton *et al.*, 2014; Bi *et al.*, 2015; Saleska *et al.*, 2016) arises, in part, due to differing assumptions about the distribution of leaf ages in forest canopies and how changes in age composition might affect ecosystem seasonality (Doughty & Goulden, 2008; Brando *et al.*, 2010; Morton *et al.*, 2014). Therefore, for such forests, 'ground truth' studies of seasonal leaf age dynamics are clearly needed.

Despite the broad interest in leaf aging, there is currently no efficient and rapid method for estimating leaf age that can be applied across forests. Previous studies linking leaf morphological development (e.g. leaf length) to leaf aging (Erickson & Miche lini, 1957; Chen *et al.*, 2009; Meicenheimer, 2014) involved laborious measurements over long time periods or relied on uncertain assumptions. Near-surface remote sensing (e.g. via 'phenocam') is an alternative technique for approximating leaf age of canopy trees in temperate deciduous forests (Richardson *et al.*, 2009; Keenan *et al.*, 2014). This approach, however, has not been tested in tropical evergreen forests and its application could prove challenging due to the high diversity of leaf phenologies, with many tree species being brevi-deciduous or evergreen during most or all of the annual cycle (Opler *et al.*, 1980; Reich, 1995; Schöngart *et al.*, 2002).

Spectroscopy may provide a fast and efficient means for estimating leaf ages from their optical properties. Differences in the reflectance, absorbance and transmittance of light at different wavelengths by plant parts are tightly coupled to their chemical composition, cell structure and physiological properties (Curran, 1989; Elvidge, 1990; Kokaly *et al.*, 2009), leading to the rapid recent development of spectroscopic methods as a general tool in plant ecophysiology and ecology. For example, spectroscopy has been used to estimate wood density and hydraulic traits (Acuna & Murphy, 2006; Petisco *et al.*, 2006; Luss *et al.*, 2015), accurately identify plant species from dried leaves (Durgante *et al.*, 2013) across developmental stages (Lang *et al.*, 2015), quantify nonstructural carbohydrate content of different plant organs (Ramirez *et al.*, 2015) and characterize a broad suite of leaf biophysical traits (Clark *et al.*, 2005; Asner & Martin, 2011; Serbin *et al.*, 2012, 2014; Asner *et al.*, 2014).

Chavana-Bryant *et al.* (2016), also in this special issue, was the first study to demonstrate that leaf reflectance spectra can successfully predict leaf age by using a partial least-squares regression (PLSR; Wold *et al.*, 2001) approach applied to data from a Peruvian evergreen forest. The underlying logic motivating the development of this spectra–age model was that because (1) leaf traits follow consistent developmental trajectories as leaves age, (2) leaf spectra emerge from the ensemble of traits that define a leaf's structure and function at any particular time (Serbin *et al.*, 2012, 2014; Asner *et al.*, 2014; Ramirez *et al.*, 2015), Thus, leaf spectra may be used directly to estimate leaf ages and, indeed, be a better predictor of leaf age than any particular limited set of leaf traits.

Although the spectra–age model was successfully tested for sunlit leaves in an evergreen forest in Peru (Chavana-Bryant *et al.*, 2016), the broader applicability and potential limitations of this approach were not explored. This study thus focuses on exploring factors that might limit the model performance, such as variation in age–trait or age–spectra relationships across forest sites and diverse canopy environments, where species composition, leaf types and trait values all vary. Specifically, we aim to answer the following questions:

(**1**) How are leaf traits and spectra related with leaf development across sites and canopy environments?

(**2**) Are these relationships sufficiently consistent to allow a general model to accurately predict leaf age from spectra across sites and various canopy environments?

In order to address these questions, we used measurements of reflectance spectra, traits and age of leaves collected at two tropical evergreen forests: we built upon the spectra–age model presented in Chavana-Bryant *et al.* (2016) that was based on sunlit leaves of a Peruvian Amazonian forest and evaluated this model at an independent Brazilian site with contrasting soil and forest properties. We then explored the consistency of relationships across both sites, with a view to developing and validating a spectra–age model generally applicable for tropical forest leaves across forest sites and canopy environments.

Materials and Methods

Study sites

The study focuses on two Amazonian evergreen forests (Fig. 1): a Brazilian site and a Peruvian site that represent contrasting edaphic and forest properties along the primary axis of ecological variation across Amazonian forests. The Brazil site is less productive, higher wood density and slower turnover but higher biomass forest than the Peru site (Malhi *et al.*, 2002, 2006; Patiño *et al.*, 2009). The contrast appears driven by soil properties, with

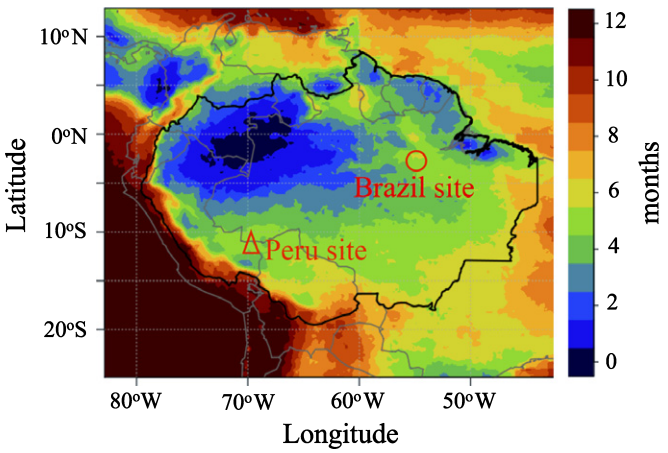

Fig. 1 Location of sites in the Amazon basin, including the Tapajos National Forest in Brazil (red circle) and the Tambopata National Reserve in Peru (red triangle). The black line indicates the boundary of the Amazon basin. The background is a map of dry season length (in months; see color legend), which is derived from Tropical Rainfall Measuring Mission (TRMM) satellite data from 1998 to 2013.

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western Amazonian soils in Peru being more fertile but with poorer physical structure (Quesada *et al.*, 2012).

The Brazil site (2°51′S, 54°58′W) encompasses the km67 eddy flux tower and associated biometric plots in Tapajos National forest, near Santarém, Brazil (Rice *et al.*, 2004; Hutyra *et al.*, 2007). Part of the Brazilian Large Scale Biosphere-Atmosphere Experiment in Amazonia (LBA) (Davidson *et al.*, 2012), this site sits on a well-drained clay-soil plateau. Mean annual precipitation is ~2000 mm yr^{-1} with a 5-month-long dry season (precipitation < 100 mm per month; Restrepo-Coupe *et al.*, 2013).

The Peru site encompasses two primary forest plots within the Tambopata National Reserve in the Madre de Dios region of Peru (Malhi *et al.*, 2014), both part of the Global Ecosystems Monitoring (GEM) network and the RAINFOR Amazon Forest Inventory Network (Malhi *et al.*, 2002), with RAINFOR codes TAM-06 (12°84′S, 69°30′W) and TAM-09 (12°83′S, 69°27′W). These forests grow on Haplic alisol soils (Quesada *et al.*, 2010), at elevations of 215 m and 220 m above sea level, respectively. Mean annual precipitation is ~1900 mm yr^{-1} (Malhi *et al.*, 2014), with a 4–5 month-long dry season (Lewis *et al.*, 2011).

Field measurements

Brazil dataset In campaigns conducted in August–September 2013, November 2013, March 2014 and July–August 2014, we selected a subset of 11 trees (Table 1) for precise leaf age monitoring. The age monitoring began with observations of leaf budburst and subsequent leaf tagging (using metal tags alongside *in situ* photos; Supporting Information Fig. S1) during the August–September 2013 campaign, when most sampled trees were flushing new leaves. Following the initial intensive tagging work, we continued to tag and photograph new leaves periodically. This age-tagging technique enabled us to accurately track leaf age in terms of days from leaf emergence at budburst (0 d) to old age (~400 d). Aside from some of the canopy leaves, this age was insufficient to sample the senescent leaf age class.

We sampled a total of 759 leaves with precise leaf age information for these 11 trees, consisting of four canopy (crowns exposed to direct sun), three mid-canopy (20–30 m tall) and four understory trees (10–20 m tall). Because we harvested both sunlit and shaded leaves for canopy trees, our dataset of precise leaf age measurements is composed of 15 tree–environment combinations: four canopy trees in a sunlit environment; four canopy trees in a shaded environment; three trees in a mid-canopy environment; and four trees in an understory environment (Table 1).

We measured reflectance spectra (see the later sub-section 'Spectral measurements in Brazil') for all 759 leaves and leaf traits (leaf mass per area, LMA; leaf water content, LWC) for a subset of 507 of these leaves which were used for the trait–age analysis reported herein. Traits were derived from leaf FW (precision at 0.001 g), area (using a Canon LiDE 120 scanner) and DW oven-dried at 60°C for 72 h.

We recorded leaf growth environments (Fig. S2), including (1) *in situ* digital hemispherical photos (collected with a 180° fisheye lens adapter for a Canon T3) to capture the radiation regime (see the later sub-section 'Within-canopy light environment'), (2) branch height (m) – the height of sampled leaves aboveground, and (3) branch depth (m) – the depth of sampled leaves below local canopy top.

In addition to the 11 trees with precisely measured leaf ages, we sampled an additional 29 tree species across diverse canopy environments, including seven canopy trees, 10 mid-canopy trees, four understory trees and eight forest-floor shrubs (< 5 m tall). The dataset included measurements of leaf traits (LMA and LWC), reflectance spectra and the canopy environments (i.e. vertical canopy positions where the leaves were harvested). This dataset did not include precise leaf ages, but provides baseline data on community level relationships between leaf traits and canopy environments for fully expanded mature leaves (Fig. S3).

Peru dataset The Peru dataset of 1072 leaves was collected in 2011, for sunlit leaves of 12 canopy trees (see Table 1 for species list). Measurements encompassed two leaf functional traits (LMA and LWC), and associated leaf reflectance spectra. Peru leaves were assigned a leaf age designed to correspond to their developmental stage, with young leaves first assigned an initial age of 1 wk when they reached a size large enough to be measured for spectra, and thereafter tracked through time until they reached advanced senescence (~400 d). Old leaves (> ~250 d) had their ages adjusted by normalization relative to maximum leaf age at senescence, taken to be 13 months (see Chavana-Bryant *et al.*, 2016; Fig. 1). Full details of the data collection and leaf age classification protocols for this site are reported in Chavana-Bryant *et al.* (2016).

We note that this method of assigning leaf ages differed from that used in Brazil, where absolute ages (based on time elapsed since tagging at emergence) were used for all leaves. This difference in age assignment methods resulted in a 1–4 wk offset in age between the datasets, depending on species (Brazil tagged leaves were measured for spectra when they were sufficiently large, typically at 2–5 wk since emergence, an age that was defined as 1 wk for Peru leaves), and a scale difference, depending on species, for old leaves (because Peru leaves were scaled to reach senescence at 13 months, whereas Brazil leaf ages were tracked to *c.* 13 months without scaling). As shown in the Results section, this difference in dating methods did not significantly limit the intercomparability of leaf age predictions between sites.

Spectral measurements in Brazil We measured leaf spectra using a full-range (350–2500 nm) FieldSpec® Pro spectroradiometer (Analytical Spectra Devices, ASD, Boulder, CO, USA). The spectrometer had a spectral sampling resolution of 1.4 nm, 2.2 nm and 2.3 nm in the visible, NIR and SWIR wavelengths respectively and all data were interpolated to 1 nm before analysis. All measurements were collected using a customized assembly attached to a plant probe with an internal calibrated light source, following Chavana-Bryant *et al.* (2016) protocols. The customized assembly was composited by two measurement blocks: one for 99.9% reflectivity white standard (Spectralon; Labsphere Inc., North Dutton, NH, USA), and the other for 3% reflectivity

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Table 1 Tree–environment combinations and associated canopy environments for leaf traits and spectra measurements at two tropical forests in Brazil and Peru

Field site	Tree–environment combination	Species	Family	DBH (m)	Canopy position	Branch height (m)	Branch depth (m)	Old LMA ($g\ m^{-2}$)	Old LWC (%)
Brazil	*E. uncinatum*_SU	*Erisma uncinatum Warm.*	Vochysiaceae	1.48	Sunlit canopy	39.0	1.0	192.60	49.63
Brazil	*E. uncinatum*_SH	*Erisma uncinatum Warm.*	Vochysiaceae	1.48	Shade canopy	30.0	10.0	130.79	48.33
Brazil	*Ocotea* sp_SU	*Ocotea* sp.	Lauraceae	0.73	Sunlit canopy	37.0	1.0	131.37	47.57
Brazil	*Ocotea* sp_SH	*Ocotea* sp.	Lauraceae	0.73	Shade canopy	32.5	5.5	91.17	45.28
Brazil	*M. huberi*_SU	*Manilkara huberi (Ducke) A. Chev.*	Sapotaceae	0.92	Sunlit canopy	37.5	0.5	229.94	49.18
Brazil	*M. huberi*_SH	*Manilkara huberi (Ducke) A. Chev.*	Sapotaceae	0.92	Shade canopy	31.2	6.8	218.25	50.39
Brazil	*C. scleroxylon*_SU	*Chamaecrista scleroxylon (Ducke) H.S. Irwin & Barneby*	Leguminosae-Caesalpinioideae	0.47	Sunlit canopy	24.5	1.0	80.47	49.79
Brazil	*C. scleroxylon*_SH	*Chamaecrista scleroxylon (Ducke) H.S. Irwin & Barneby*	Leguminosae-Caesalpinioideae	0.47	Shade canopy	20.0	5.5	59.31	55.74
Brazil	*E. uchi*_MC	*Endopleura uchi (Huber) Cuatrec.*	Humiriaceae	0.46	Mid-canopy	24.5	16.5	114.19	46.01
Brazil	*E. coriacea*_MC	*Eschweilera coriacea*	Lecythidaceae	0.25	Mid-canopy	22.8	15.2	122.01	44.63
Brazil	*H. courbaril*_MC	*Hymenaea courbaril L.*	Leguminosae-Caesalpinioideae	0.45	Mid-canopy	30.0	11.0	117.21	52.02
Brazil	*Miconia* sp_US	*Miconia* sp.	Melastomataceae	0.14	Understory	13.7	29.3	58.62	61.37
Brazil	*G. amazonicum*_US	*Glycydendron amazonicum (Ducke)*	Euphorbiaceae	0.11	Understory	7.7	33.3	54.74	62.96
Brazil	*M. ruficalyx*_US	*Miconia ruficalyx Gleason*	Melastomataceae	0.14	Understory	13.5	12.0	54.73	59.80
Brazil	*V. elongate*_US	*Virola elongata (Benth.) Warb.*	Myristicaceae	0.17	Understory	19.0	6.5	75.65	59.84
Peru	*L. brittoniana*_SU	*Licania brittoniana*	Chrysobalanaceae	1.88	Sunlit canopy	29.5	0.5	121.09	49.89
Peru	*Q. simaruba*_SU	*Quassia simaruba*	Simaroubaceae	1.62	Sunlit canopy	29.0	0.5	188.04	44.49
Peru	*R. ovale*_SU	*Ruizodendron ovale*	Annonaceae	1.23	Sunlit canopy	32.6	0.5	91.27	50.53
Peru	*A. parvifolium*_SU	*Aspidosperma parvifolium*	Apocynaceae	1.57	Sunlit canopy	33.7	0.5	110.94	55.09
Peru	*C. macrosperma*_SU	*Couratari macrosperma*	Lecythidaceae	1.43	Sunlit canopy	33.7	0.5	170.65	45.78
Peru	*L. longistyla*_SU	*Licania longistyla*	Chrysobalanacea	1.43	Sunlit canopy	30.3	0.5	125.87	37.70
Peru	*B. excelsa*_SU	*Bertholletia excelsa*	Lecythidaceae	2.30	Sunlit canopy	34.0	0.5	147.93	45.07
Peru	*T. chrysaloides*_SU	*Tachigali chrysaloides*	Caesalpinioideae	2.10	Sunlit canopy	29.7	0.5	180.51	47.29
Peru	*C. racemosa*_SU	*Clarisia racemosa*	Moraceae	1.58	Sunlit canopy	33.8	0.5	120.86	49.76
Peru	*E. coriacea*_SU	*Eschweilera coriacea*	Lecythidaceae	1.88	Sunlit canopy	33.3	0.5	157.55	46.61
Peru	*G. boliviana*_SU	*Gautteria boliviana*	Annonaceae	1.17	Sunlit canopy	31.9	0.5	113.31	52.22
Peru	*P. franciscana*_SU	*Pouteria franciscana*	Sapotacea	2.37	Sunlit canopy	35.4	0.5	124.72	48.31

Tree–environment combinations were coded by *'species name_canopy position'*, with four canopy position codes: SU, sunlit; SH, shaded; MC, mid-canopy; US, understory. DBH, diameter at breast height; LMA, leaf mass per area; LWC, leaf water content; Old: leaf age > 300 d (and senescent leaves were excluded).

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dark standard (Odyssey III black 449/9009 Marine Grade Cover Fabric). For each leaf, reflectance spectra were measured on 1–6 different parts of the leaf adaxial surface and then averaged to determine the mean optical properties across all wavelengths.

Vegetation indices (VIs) In order to include important aspects of leaf bio-physiological traits that are not fully covered by LMA and LWC, we calculated four commonly used VIs, including Normalized Difference Vegetation Index (NDVI; Eqn 1; Tucker, 1979; Ustin *et al.*, 2009), Enhanced Vegetation Index 2 (EVI2; Eqn 2; Jiang *et al.*, 2008), Photosynthetic Reflectance Index (PRI; Eqn 3; Gamon *et al.*, 1992) and Normalized Difference Water Index (NDWI; Eqn 4; Gao, 1996).

$$\mathrm{NDVI} = \frac{\mathrm{NIR} - R}{\mathrm{NIR} + R} \qquad \text{Eqn 1}$$

$$\mathrm{EVI2} = 2.5 \times \frac{\mathrm{NIR} - R}{\mathrm{NIR} + 2.4 \times R + 1} \qquad \text{Eqn 2}$$

$$\mathrm{PRI} = \frac{\rho_{531} - \rho_{570}}{\rho_{531} + \rho_{570}} \qquad \text{Eqn 3}$$

$$\mathrm{NDWI} = \frac{\mathrm{NIR} - \mathrm{SWIR}}{\mathrm{NIR} - \mathrm{SWIR}} \qquad \text{Eqn 4}$$

(NIR, reflectance at near-infrared 800 nm band; R, reflectance at Red 680 nm band; ρ_{531}, reflectance at 531 nm band; ρ_{570}, reflectance at 570 nm band; SWIR, reflectance at short-wavelength infrared 1240 nm band).

These VIs represent important leaf bio-physiological properties: NDVI and EVI2 are the integrated metric for the greenness and structure of leaves (Sellers *et al.*, 1992; Huete *et al.*, 2002); PRI is a measure of the intrinsic quantum yield for photosynthesis (Gamon *et al.*, 1992); NDWI is an indicator of leaf water content or hydrological status (Gao, 1996). By using these VIs (together with leaf traits of LMA and LWC, and spectra), we aim for a more comprehensive understanding of the effect of canopy environments on leaf properties and their developmental trajectories.

Within-canopy light environment We estimated the within-canopy light environment from *in situ* digital hemispheric photos (see the earlier sub-section 'Brazil dataset'). These photos were preprocessed and quality controlled using Adobe LIGHTROOM 4 (Adobe Systems Inc., San Jose, CA, USA). Contrast was then optimized and the modified images were exported in JPEG format. Using a custom MATLAB program together with Otsu's algorithm (Otsu, 1975), these images were automatically binarized into sky (or gap) or non-sky pixels. The image fraction of the sky, or gap fraction, was then calculated to index the light environment of the leaf sample.

Spectra–age modelling

General approach As in Chavana-Bryant *et al.* (2016), we used the partial least-squares regression (PLSR) modeling approach (Geladi & Kowalski, 1986; Wold *et al.*, 2001), which was adapted from several recent studies (Wolter *et al.*, 2008; Serbin *et al.*, 2014; Singh *et al.*, 2015). PLSR is the current state-of-the-art approach for linking leaf and canopy spectroscopy with leaf and plant traits (e.g. Bolster *et al.*, 1996; Townsend *et al.*, 2003; Asner & Martin, 2011; Serbin *et al.*, 2014). Previous studies have also shown that PLSR is a more robust method compared to simple correlation or multiple linear regression approaches (Geladi & Kowalski, 1986; Grossman *et al.*, 1996; Wold *et al.*, 2001).

Here the PLSR included five steps (Fig. S4): (1) filtering of outliers (which removed ~5% of data) following the Monte-Carlo sampling method for outlier detection (Xu & Liang, 2001); (2) the filtered dataset was one-time randomly divided into the training (70%) and testing (30%) datasets; (3) 90% of the training dataset was randomly selected (with 100-time replication) for PLSR analysis, with the latent variable number varying from 1 to n ($n = 20$ in our case); (4) the PLSR regression coefficients were applied to the training and testing datasets, with model performance assed by using root mean squares error (RMSE), and R^2 (the proportion of variance of observation explained by model); (5) the optimal latent variable numbers were then identified by minimizing RMSE and maximizing R^2 for the testing dataset.

We implemented the above PLSR analysis to our predictor variables, using the MCS function from LIBPLS (http://www.libpls.net) for outlier removal, PLSREGRESS function in MATLAB (Mathworks, Natick, MA, USA) for the PLSR analysis, and custom MATLAB functions for other steps. The predictor variables in this study can be either leaf spectra only (400–2500 nm; see 'Peru Spectra model' in the next sub-section and 'All Spectra model' in the later sub-section 'Generalizing the leaf age model across canopy environments') or leaf spectra combined with leaf traits (see 'Peru Spectra+all Trait model' and 'All Spectra+all Trait model' in the later sub-section 'Generalizing the leaf age model across canopy environments').

Cross-site spectra–age analysis We first examined the ability to model leaf age from leaf spectra across different sites through a series of tests. We used the spectra–age model developed for Peruvian sunlit leaves (Chavana-Bryant *et al.*, 2016) as a 'reference model' (or 'original Peru Spectra model'), applying it to the Brazilian dataset which included leaves sampled from four canopy environments. The goal was to explore the potential for generalizing the spectra–age model across sites (from Peruvian sunlit leaves to Brazilian sunlit leaves) and across canopy environments (from Peruvian sunlit leaves to Brazilian shade canopy, middle-canopy and understory leaves).

Generalizing the leaf age model across canopy environments Because leaf growth environments affect within-canopy leaf trait variation (Ellsworth & Reich, 1993; Cavaleri *et al.*, 2010), we expected that the leaves from the broader range of growth environments in Brazil would have different optical properties (and therefore different relationships between leaf spectra and age) as compared with the sunlit leaves from Peru

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(Chavana-Bryant *et al.*, 2016). To investigate how spectra–age relationships depend on the different growth environments and their associated traits and, hence, to develop a more general model of leaf age applicable across these growth environments, we first conducted a reference test ('Test 0') of how well the original Peru Spectra model predicted leaves across different sites and environments. We then tested three models of leaf age–trait–spectra relationships across canopy environments:

– Test 1 (Peru Spectra+ all Trait model): determines whether accounting for changing growth environments and their associated leaf traits could improve performance of the Peru reference model when applied to leaves from all environments. To this end, we used the original leaf spectral variables for the Peru reference model alongside the added variables, including branch height, depth, LMA, LWC and four VIs, as the new predictor variables, to generate a 'Peru Spectra+all Trait' PLSR model.

– Test 2 (All Spectra model): tests whether a more general spectra-only model can predict leaf ages across all categories of leaves and growth environments. In this test, we used combined Peru and Brazil datasets (with leaves encompassing the full range of traits that emerge from development under different environments) to re-parameterize an 'All Spectra' model (with no traits included explicitly).

– Test 3 (All Spectra+all Trait model): a simple combination of Tests 1 and 2.

We hypothesized (H1) that including traits and proxies for growth environments (Test 1) would indeed improve model generality from sunlit leaves to understory leaves. A positive outcome for Test 1 (which would show that accounting for environmental influence on leaf trait variation improves ability to predict age) would suggest a second hypothesis (H2) that a spectra-only model (Test 2) should be able to perform as well as, or even better than, the hybrid model of Test 1. This is because spectral models have been shown to predict a broad array of traits (e.g. Serbin *et al.*, 2014), including traits that are unmeasured for the leaves used in this study but which may also be associated with age (Chavana-Bryant *et al.*, 2016). Finally, we hypothesized (H3) that Test 3 will perform only marginally better than Test 2, because the spectra will themselves already capture the majority of the variation in the response as compared to including traits separately in the model.

In order to test these hypotheses, in addition to the two metrics of model goodness, RMSE and R^2, we also calculated the Akaike Information Criterion (AIC) for the model cross-comparisons. The AIC used is formatted as $\mathrm{AIC} = N \times \log(\delta^2) + 2 \times m$, following the literature (Akaike, 1974; Aho *et al.*, 2014), where N is the number of leaves, δ is RMSE, and m is the optimal latent variable number for each PLSR modeling scenario.

Results

We first focus on the results from the Brazil site (reported here for the first time), and then show integration with the Peru dataset (from Chavana-Bryant *et al.*, 2016).

Leaf traits and spectra vary with age across canopy environments and forests

Despite the broad trait variation induced by different canopy environments (from full sun to deeply shaded understory environment, Table S1), correlations of leaf traits LMA and LWC with leaf age were evident across the Brazilian site community ($R^2 = 0.20$, $P < 10^{-5}$ for LMA; $R^2 = 0.42$, $P < 10^{-5}$ for LWC; Fig. 2a,b). The trait differences across canopy environments tended to obscure the strength of these correlations, which were more evident within specific canopy environments across all trees ($R^2 = 0.23$–0.72 for LMA and $R^2 = 0.60$–0.80 for LWC; Fig. 2) and within environments of individual trees. Within individual trees, all 11 tree–environment combinations showed a significantly positive trend in LMA–age relationships ($R^2 = 0.52$–0.91), and significantly negative LWC–age relationships ($R^2 = 0.60$–0.95). These positive LMA–age and negative LWC–age relationships are consistent with those that were observed at the contrasting forest in Peru where a single leaf environment was sampled (sunlit leaves, Chavana-Bryant *et al.*, 2016).

Spectral data also showed strong dependency on leaf age and leaf canopy environments across all Brazilian tree–environment combinations (Figs 3, S5). Mean visible reflectance, especially the

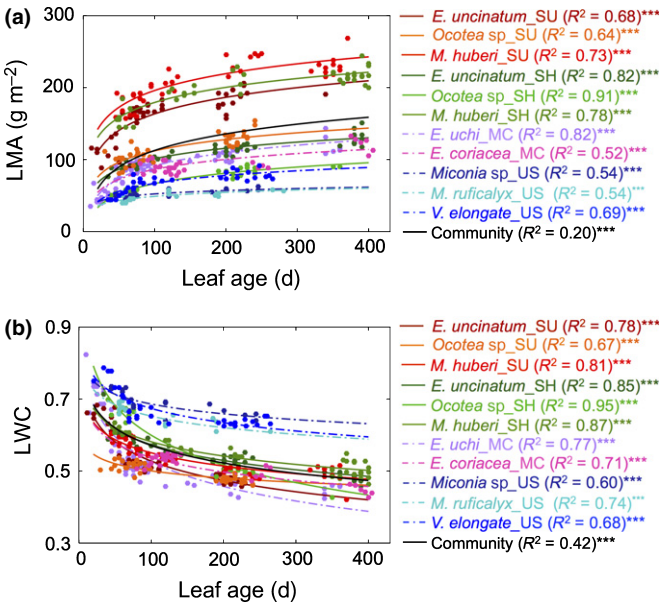

Fig. 2 Leaf trait variation with leaf age and canopy environments at the Brazil site for 11 tree–environment combinations (see Table 1). (a) Leaf mass per area (LMA) and (b) leaf water content (LWC). Each colored line represents a tree in a particular environment (indicated by a *'species name_canopy position'* label in the legend, where 'canopy position' is represented by four codes: SU, sunlit canopy; SH, shade canopy; MC, mid-canopy; US, understory). Solid colored lines indicate sunlit canopy environment ($R^2 = 0.31$ for LMA; $R^2 = 0.60$ for LWC) and shade canopy environment ($R^2 = 0.23$ for LMA; $R^2 = 0.80$ for LWC), dashed lines indicate mid-canopy (MC, $R^2 = 0.76$ for LMA; $R^2 = 0.74$ for LWC) or understory (US, $R^2 = 0.39$ for LMA; $R^2 = 0.61$ for LWC), and black lines indicate community average relationships: R^2 is the proportion of variation in the trait that is explained by leaf age (model: $\log(\text{trait}) = a \times \log(\text{age}) + b$). ***, $P < 10^{-5}$.

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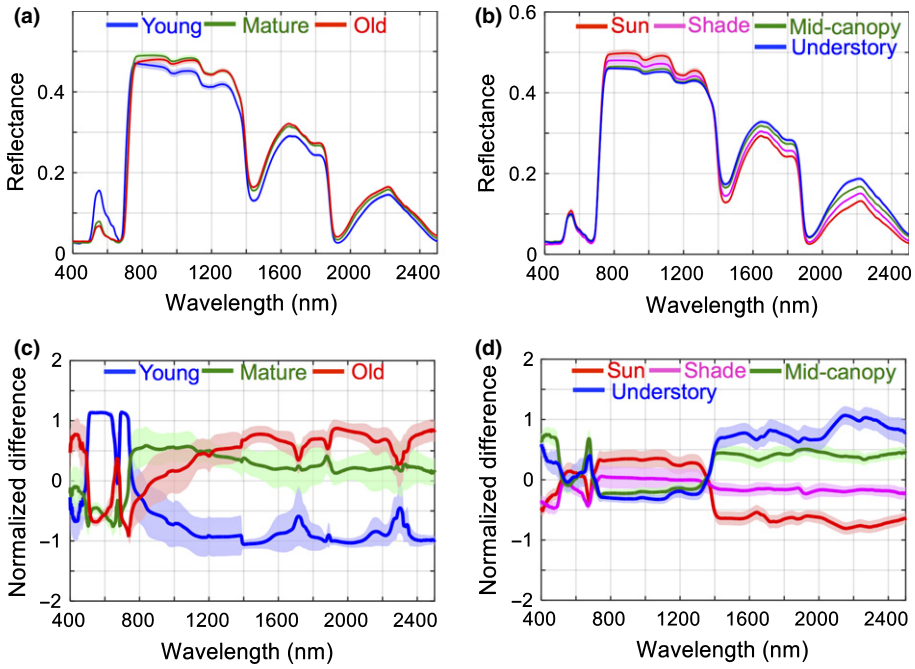

Fig. 3 Leaf age and canopy environments induced spectra variation at the Brazil site. (a) Age-dependent leaf level hyperspectral reflectance across all canopy environments for: young, mature and old leaves (mean value in solid lines with shaded 95% confidence interval). (b) Canopy environment-dependent leaf level hyperspectral reflectance across all leaf ages for: sunlit canopy ($n = 224$ leaves), shade canopy ($n = 207$), mid-canopy ($n = 186$) and understory ($n = 142$). (c) Normalized differences of young, mature, and old leaf spectra from the mean leaf spectra (solid lines ± shaded 95% confidence interval). (d) Normalized environment differences of sunlit canopy, shade canopy, mid-canopy and understory leaf spectra from the mean leaf spectra (solid lines ± shaded 95% confidence interval). Normalized difference = (mean reflectance within each scenario − mean reflectance across all scenarios)/standard deviation of reflectance across all scenarios.

green peak (~ 550 nm), and its variance showed continuous declines with age across all Brazilian tree–environment combinations. Initial mean NIR reflectance (800–1200 nm) increased (with lower variance) during leaf expansion, which was followed by decreases in the mean (and increases in variance) as leaves aged (Fig. 3a,c). Mean SWIR reflectance (1400–2500 nm) increased monotonically with age, whereaas the variance initially decreased and then increased as leaves aged. These patterns of the relative spectra change with leaf age, observed at the Brazil site across vertical canopy profiles, are also consistent with those observed in the Peruvian sunlit leaves (Chavana-Bryant *et al.*, 2016).

Strong spectral dependencies on canopy environments were also observed (Fig. 3b). Upper canopy vs middle canopy or understory differences were especially strong in the SWIR, where reflectance increased monotonically with depth into the canopy (Fig. 3d). Effects due to canopy environments were comparable to those of leaf age, indicating that models intended to predict age across different canopy environments would likely need to account for growth environment effects.

Cross-site spectra–age analysis

Because leaf traits and spectra consistently vary with leaf age across both canopy environments and forest sites (albeit with offsets among the different canopy environments), we explored the application of the Peru-trained spectra–age model to the independent forest in Brazil. We found that the Peruvian model developed in Chavana-Bryant *et al.* (2016, with seven latent variables) predicted ages of leaves for the Brazilian canopy trees with high precision ($R^2 = 0.75$ and $R^2 = 0.78$ for sunlit and shade canopy leaves, respectively) (see Fig. 4a,b at seven latent variables). However, model performance when predicting ages of leaves from middle and understory Brazilian trees was poor ($R^2 = 0.27$ and $R^2 = 0.29$ for Brazilian middle-canopy and understory leaves, respectively) (Fig. 4a,b for seven latent variables).

We sequentially re-fitted the Peru-trained model with different numbers of latent variables, in order to investigate whether there existed an optimum number of latent variables that would improve the Peru-trained model performance across the range of canopy environments at the Brazil site. We found that reducing the number of latent variables from seven to five significantly improved performance of the Peru-trained model in predicting the ages of Brazil middle-canopy and understory leaves, without resulting in a significant reduction in performance for Peruvian sunlit leaves (for which the seven-variable model was optimal) (Fig. 4a,b). We thus adopted the five-variable model as the Peru reference model, optimized across canopy environments.

Closer investigation of the performance of the seven-variable Peru-trained spectra–age model for individual Brazilian trees revealed that the relatively poor performance of this model relative to the five-variable model was confined to early developing leaves (≤ 40 d old) of one middle-canopy and two understory trees (*E. uchi*_MC, *G. Amazonicum*_US, and *M. ruficalyx*_US; Table 1 and Fig. 5). The early developing leaves of these trees exhibited 'reddish' coloration (e.g. Fig. 5a), a common early developmental process displayed by sub-canopy leaves but not by canopy sunlit leaves, which has confounding effects for the seven-variable Peru model (Fig. 5c).

The PLSR regression coefficients (Fig. 4c) and Variable Importance in Projection (VIP; Fig. 4d) from this optimized reference model indicated the important spectral domains responsible for leaf age modeling, which included visible domain (especially ~ 550 nm), red edge (~ 725 nm), NIR (~ 800 nm) and several water absorption bands (~ 1440 nm, ~ 1700 nm and ~ 1920 nm). These patterns also matched well with the age-dependent spectral

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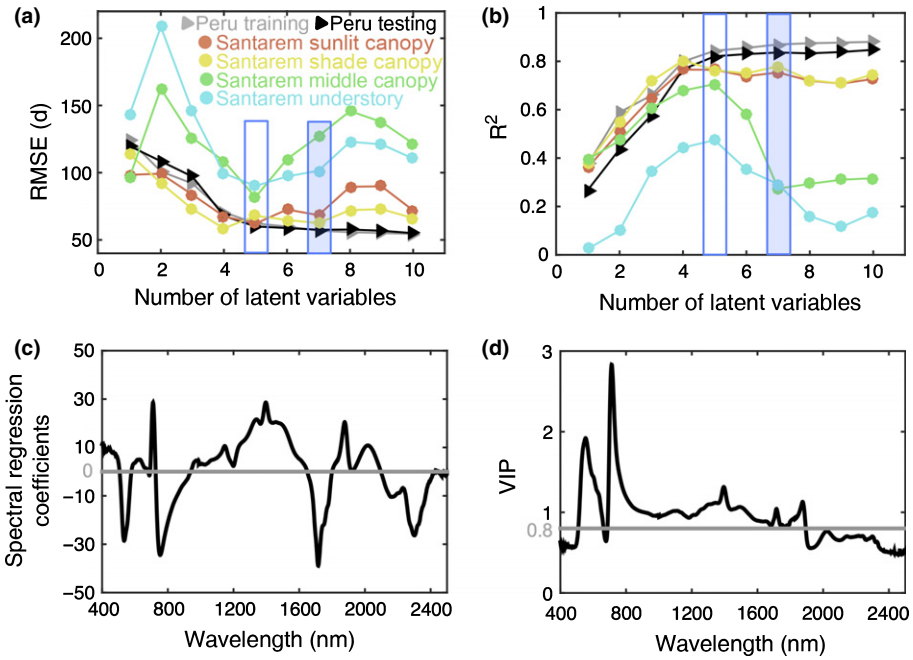

Fig. 4 Cross-site spectra–age model results for data from both Peruvian and Brazilian sites, based on fitting variation of the Peru reference partial least-squares regression (PLSR) models to a subset of observations at the Peru site only (i.e. the Peru training dataset). (a) Root mean square error (RMSE) between observed and modeled leaf age plotted against the number of latent variables incorporated for PLSR models. (b) The proportion of variation in leaf age explained by PLSR models (R^2) plotted against the number of latent variables incorporated. Different symbols in (a) and (b) represent different datasets, as indicated (see also Table 1): the performance of the original seven-latent variable model for each dataset is indicated by the points in the blue shaded box in a, b); an optimal model for prediction across sites (RMSE minimized and R^2 maximized for Brazil validation datasets not used in model fitting) emerges for five latent variables (blue box in a, b). (c) Spectral regression coefficients for the optimized PLSR model with five latent variables. (d) Variable importance in projection (VIP) for the optimized PLSR model with five latent variables (spectral features > 0.8 represent the important spectral regions for leaf age modeling).

variation (Fig. 3a,c), providing confidence for our spectra–age modeling.

The optimized Peru model performance using the Peru testing data was $R^2 = 0.83$ and RMSE = 55 d compared with $R^2 = 0.64$ and RMSE = 72 d when applied to all Brazilian data (Table S2). When the Brazil data were separated by canopy environments, cross-site performance of the Peru model applied to the Brazil data ranged from high performance in sunlit and shade canopy leaves and middle-canopy leaves ($R^2 = 0.77$, RMSE = 62 d for sunlit, and $R^2 = 0.77$, RMSE = 62 d for shade canopy leaves, and $R^2 = 0.71$, RMSE = 80 d for middle-canopy leaves; Fig. 4 and Table S2) to lower performance when applied to understory leaves ($R^2 = 0.29$, RMSE = 101 d for the original Peru model and $R^2 = 0.47$, RMSE = 90 d for the optimized Peru model) (Fig. 4). In sum, we found that the greater the disparity in canopy environment from that used to train the Peru reference model, the larger the inaccuracies in leaf age model predictions.

The range of leaf traits affects cross-site model generality

We investigated whether the relatively lower performance of the Peru-trained canopy sunlit leaf age model for leaves sampled from canopy environments beyond its original scope was associated with different suites of traits and/or developmental pathways not included in the reference model training dataset, and if so, whether such differences were linked systematically to the broader range of canopy environments. We found that sunlit canopy leaves from the two sites largely overlapped in both their height above the ground (a proxy for growth environment), and in their trait values, but that sunlit leaves were significantly different from understory leaves in growth-environments and leaf trait values for old leaves (Fig. 6). The presence of 'reddish' early developmental leaves in the middle canopy and understory also demonstrates the existence of a different developmental pathway. This environmentally driven divergence in traits (as also seen in Ellsworth & Reich, 1993; Cavaleri *et al.*, 2010) provides a mechanistic basis for improving predictive models of leaf age applicable across canopy environments.

In order to leverage this result, we quantified how model fit of the optimized Peru reference model (developed for Peruvian sunlit leaves) depended on different environments. Deviations of predictions from observations were characterized by simple linear regressions for each tree–environment combination (as seen by regression lines between model predicted and observed ages; Fig. 7). These regression lines showed systematic deviation from the 1 : 1 line, with strong dependency on leaf growth environments: Deviations in canopy sunlit environments (Fig. 7a–d) usually (three of four trees) followed a slope shallower than the 1 : 1 line, whereas at the other end of the environmental gradient, deviations in Brazilian understory trees were significantly steeper than the 1 : 1 line (Fig. 7l–o). Deviations were largest in understory environments, whose leaf ages tended to be significantly

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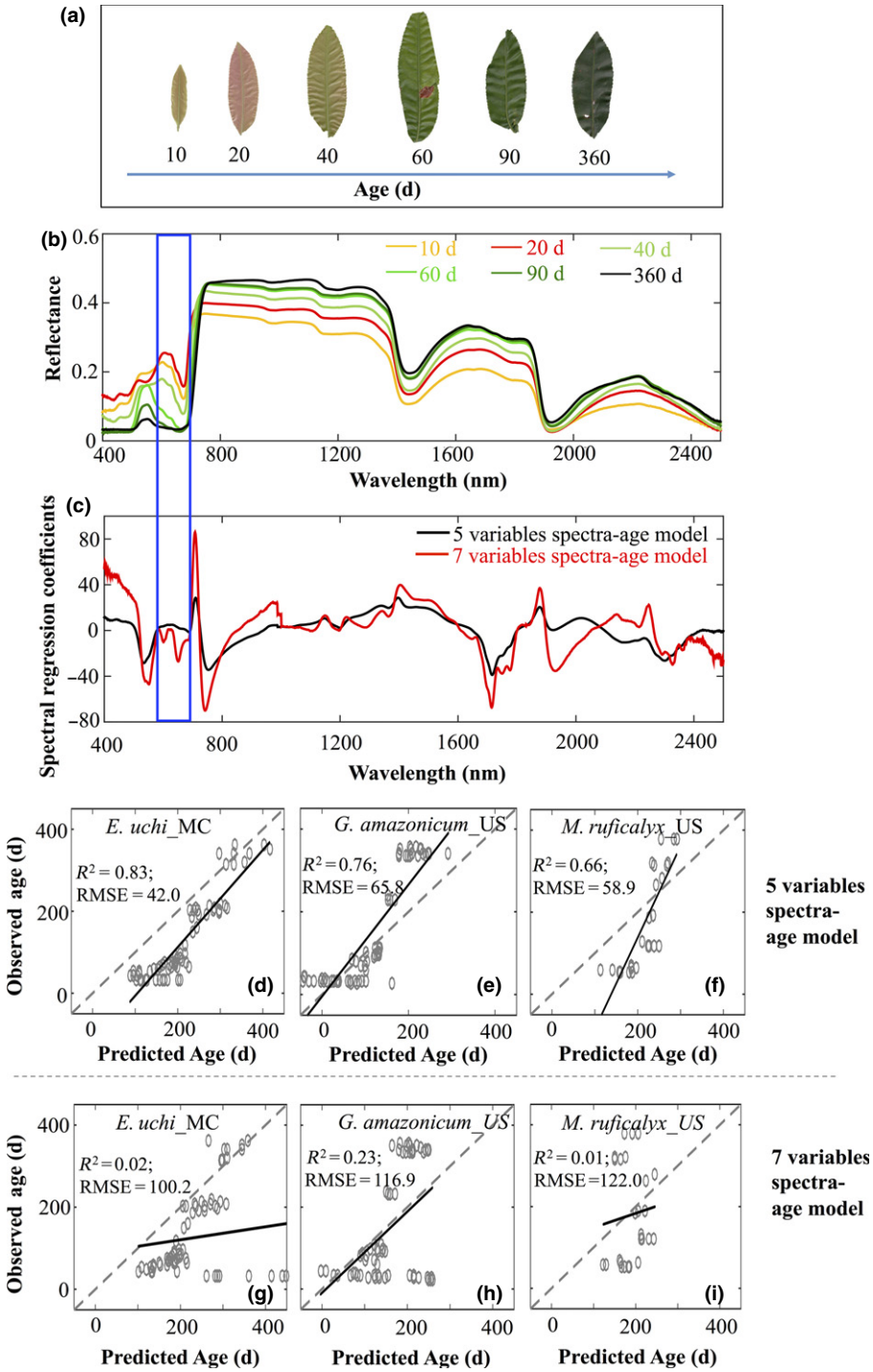

Fig. 5 Example of developmental trajectory in a mid-canopy tree in Brazil (*E. uchi*_MC), including: (a) the appearance of leaves vs age in RGB photos, showing reddish leaves when leaf ages are 40 d or younger; (b) the aging of leaves as revealed by leaf reflectance hyperspectra (measured by an ASD spectradiometer); and (c) comparison between spectra–age model coefficients of seven latent variables (in red) and five latent variables (in black), (see Fig. 4; as derived from the model parameterized by Peruvian sunlit canopy leaves, which do not have reddish young leaves). Coefficients in the spectral region marked by the blue window are near-zero in the five-variable model, which more accurately predicts leaf age in three mid-canopy or understory trees: (d) *E. uchi*_MC, (e) *G. amazonicum*_US and (f) *M. ruficalyx*_US). The nonzero coefficients in the seven-variable model make it more sensitive to reddish shifts in the understory Brazilian leaves, a confounding effect which causes significant overprediction of young leaf age in these same trees when the seven-variable model is applied (in g–i). See Table 1 for full species names.

overestimated in young age classes, but underestimated in old age classes.

We found that the deviation of leaf age model performance could be tied to canopy environments (and to leaf traits): the deviations, as represented by the variation in the parameters (slope and intercept) of lines fit to those deviations, were explained systematically by canopy environments, as captured by branch height (Fig. 8a,c), and by traits (e.g. LMA, Fig. 8b,d). Therefore, variability in canopy environments and traits is the source of the lower performance of the optimized Peru leaf age model when extended to new canopy environments. This suggests that modeling strategies that account for variation in traits or proxies for canopy environment (e.g. branch height) should produce more general models of leaf age that are applicable across different canopy environments.

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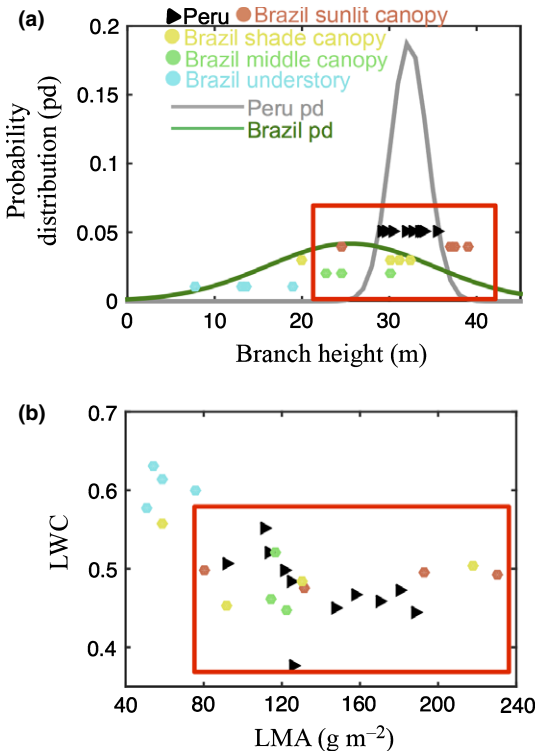

Fig. 6 (a) Branch height probability distribution for Peru and Brazil, with color symbols indicating branch height for each tree-environment combination. (b) Leaf trait scatter plot showing leaf water content (LWC) vs leaf mass per area (LMA) for Peru and Brazil leaf samples from the old leaf age class (Table 1). Red rectangles in (a) and (b) bound the sample space for mid- to upper canopy leaves from Peru and Brazil sites.

Generalizing the leaf age model across canopy environments

Four modeling exercises were conducted to explore model generality across canopy environments in Brazil (Fig. 9), with the optimum five latent variables for 'Peru Spectra' and 'All Spectra' models and the optimum six latent variables for 'Peru Spectra+all Trait' and 'All Spectra+all Trait' models (Fig. S6). The calculated AIC metric for each modeling scenario showed a consistent, positive relationship with RMSE, suggesting that RMSE is a good metric for the cross-model comparison in this study (Table S2). Relative to the optimized Peru reference model (Fig. 9b), the 'Peru Spectra+all Trait' model, which incorporated the covariates of growth environments and leaf traits, modestly improved overall performance for all the Brazil data ($R^2 = 0.69$, RMSE = 74 d vs $R^2 = 0.64$, RMSE = 72 d for reference; Fig. 9c), but significantly improved prediction for Brazilian understory samples (R^2 increased from 0.47 to 0.57; RMSE decreased from 90 d to 88 d).

The 'All Spectra' model, parameterized by both Peruvian and Brazilian leaf spectra (Fig. 9d), achieved large performance gains across canopy environments (R^2 increased from 0.64 to 0.79 and RMSE decreased from 72 d to 53 d for all Brazilian leaves; R^2 increased from 0.47 to 0.73 and RMSE decreased from 90 d to 72 d for Brazilian understory leaves).

The 'All Spectra+all Trait' model, parameterized by both Peruvian and Brazilian leaf spectra and traits (Fig. 9e), led to the best model overall, but, as we hypothesized (H3 from earlier subsection 'Generalizing the leaf age model across canopy environments), it gave only a modest improvement over the 'All Spectra' model overall (R^2 increased from 0.79 to 0.81 and RMSE decreased from 53 d to 50 d; Fig. 9). Specifically, the most significant improvement occurred in Brazilian understory leaves (R^2 increased from 0.73 to 0.82 and RMSE decreased from 72 d to 60 d; Fig. 9), at the small expense of model performance for Brazilian canopy shade leaves (R^2 decreased from 0.89 to 0.88 and RMSE increased from 48 d to 49 d; Fig. 9).

Discussion

We investigated whether principles of leaf trait ecology and ontogeny could be used to create a general model relating leaf spectra to leaf age, taking into account the effect of canopy environments. We divided this investigation into two broad questions: how are leaf traits and spectra related to leaf development across different sites and canopy environments? Are these relationships sufficiently consistent to allow a general model to accurately predict leaf age from spectra across sites and various canopy environments?

How are leaf traits and spectra related to leaf development across different sites and canopy environments?

Two key findings address this question:

(**1**) Variation in leaf traits and spectra across all leaves is large compared to datasets that focus only on sunlit mature leaves. Our 759 leaves from 11 trees in Brazil encompassed variation in leaf mass per area (LMA; 35–270 g m^{-2}), leaf water content (LWC; 42–83%) and near infrared (NIR) reflectance (0.35–0.64) (Table S1) that covers over 98% of LMA values and 89% of NIR reflectance values recorded for the much larger dataset of 1449 tree species (6136 leaves) in Asner *et al.* (2011, 2014). These results are consistent with those reported at the contrasting forest site with very different soil condition (Quesada *et al.*, 2012) in Peru (Chavana-Bryant *et al.*, 2016) and show that such large variation in traits and spectra can be attributed primarily to the substantial variation across leaf ages (Figs 2, 3) and canopy environments (Figs 2, 3, S3).

This finding highlights two important points: first, it emphasizes how leaf age (Hulshof *et al.*, 2013; Chavana-Bryant *et al.*, 2016) and canopy environments (Wright *et al.*, 2004; Asner *et al.*, 2011, 2014; Serbin *et al.*, 2014) can be key drivers of trait variation that cause within-species traits to vary as much or more than variations across species; and second, most relevant for this study, it confirms that if leaf age varies in concert with leaf traits and spectra, then sampling leaves for a broad range of traits (and how they vary with leaf age and canopy environments) may be more important than sampling many sites or species in developing a general model for predicting leaf age from spectra.

(**2**) Leaf traits and spectra vary with both age and canopy environments (Figs 2, 3, 6, S3). Previous studies have found that leaf

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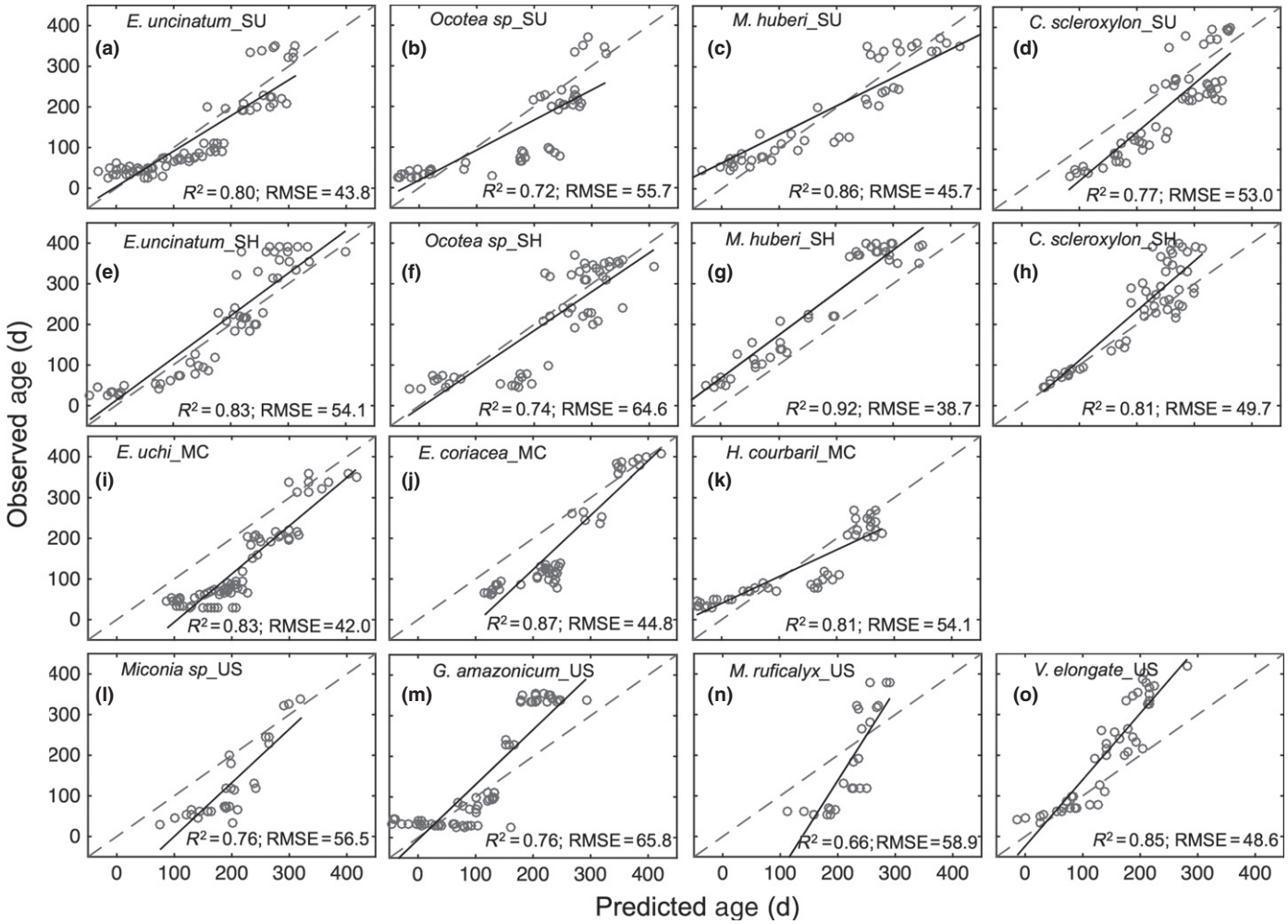

Fig. 7 Performance (observed vs predicted leaf age) of the optimal Peru-trained spectral leaf age model (Fig. 4, five-variable model) as applied to Brazilian samples from four canopy environments: (a–d) sunlit canopy; (e–h) shade canopy; (i–k) mid-canopy; (l–o) understory. OLS regressions (black lines) quantify the deviation of the scatterplots from the ideal 1 : 1 line (dashed lines). R^2 quantifies the fit of the regression line – i.e. the variation in leaf age explained by combining the optimized Peru leaf age model with the individual tree regressions – and RMSE is the corresponding root mean square error. See Table 1 for full species names.

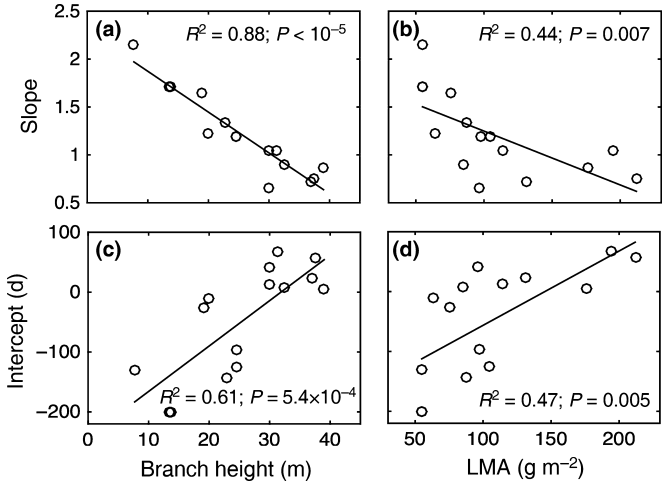

Fig. 8 The regression line slopes and intercepts of each tree–environment combination (from Fig. 7) plotted against branch height and leaf mass per area (LMA) for the Brazil site: (a) slope vs branch height; (b) slope vs LMA; (c) intercept vs branch height; (d) intercept vs LMA.

age influences leaf traits and spectra (Field & Mooney, 1983; Kitajima *et al.*, 1997, 2002; Roberts *et al.*, 1998; Wilson *et al.*, 2001; Yang *et al.*, 2014; Chavana-Bryant *et al.*, 2016) and that canopy environments influence leaf traits (e.g. Lichtenthaler *et al.*, 1981; Givnish, 1988; Ellsworth & Reich, 1993; Koike *et al.*, 2001; Kumagai *et al.*, 2001; Terashima *et al.*, 2001; Cavaleri *et al.*, 2010; Coble & Cavaleri, 2015; Kenzo *et al.*, 2015), but this study also finds that understanding their interaction (Figs 2, 3a,b) is particularly important for developing general relationships between leaf ages and trait-mediated spectra.

These interacting trait–age and trait–environment relationships lay the foundation for addressing our second question (below). This is because the age-dependent and/or environment-dependent changes in the abovementioned leaf traits and other related leaf morphological, structural and physico-chemical traits are known to influence leaf optical properties (Curran, 1989; Elvidge, 1990; Jacquemoud & Baret, 1990; Carter, 1993; Kokaly *et al.*, 2009; Asner *et al.*, 2011, 2014; Serbin *et al.*, 2012, 2014;

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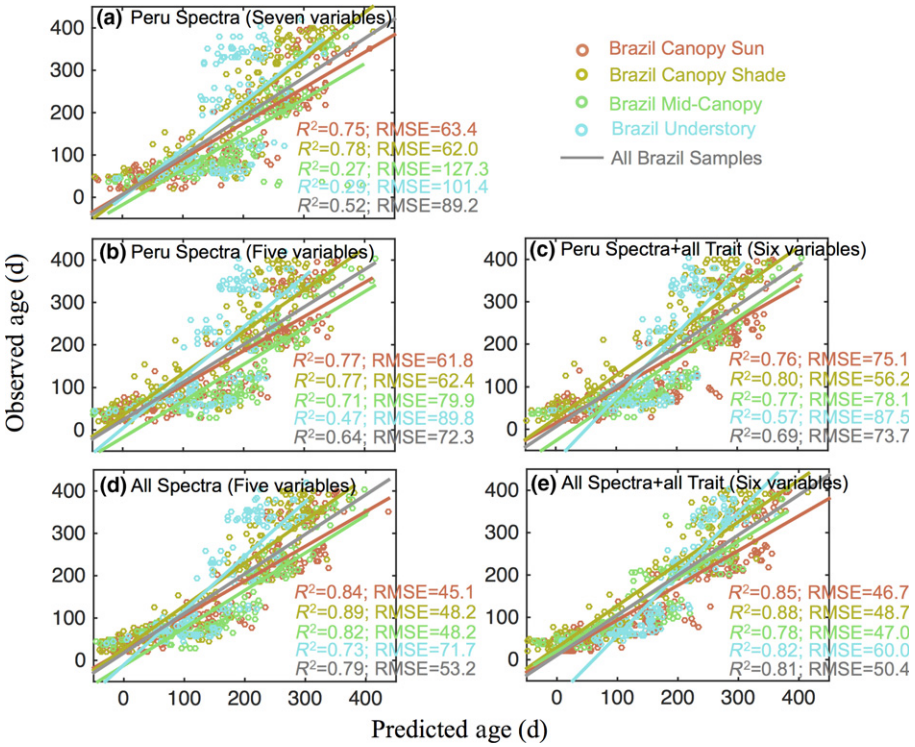

Fig. 9 Performance of leaf age models for the Brazilian leaf samples under five scenarios: (a) the Peru model (parameterized by using Peruvian leaf spectra only; the same model as presented in Chavana-Bryant *et al.*, 2016 using seven latent variables); (b) the optimized Peru reference model (parameterized by using Peruvian leaf spectra only); (c) the 'Peru Spectra+ all Trait' model (parameterized by using Peruvian leaf spectra and traits); (d) the 'All Spectra' model (parameterized by using both Brazilian and Peruvian leaf spectra); (e) the 'All Spectra+ all Trait' model (parameterized by using both Brazilian and Peruvian leaf spectra and traits). Four different color circles represent the leaf samples from Brazil sunlit canopy ($n = 4$ trees), Brazil shade canopy ($n = 4$), Brazil mid-canopy ($n = 3$) and Brazil understory trees ($n = 4$). Four different color lines represent the corresponding ordinary least regression (OLS) between predicted and observed leaf ages; central grey line represents the OLS analysis for all Brazil samples. The 'All Spectra' model (d) is our 'recommended' general model. The number of optimal latent variables in (b) was identified in Fig. 4, and in (c–e) were identified in Supporting Information Fig. S6.

Yang *et al.*, 2014; Chavana-Bryant *et al.*, 2016). Intriguingly, the spectrally based species identification study of Lang *et al.* (2015) noted a result that leaves from young and adult plants differed consistently in their NIR spectra, which parallels what we investigated here (and also what is reported in Chavana-Bryant *et al.*, 2016) across the developmental stages of individual leaves within adults. This suggests that, ultimately, the spectra–age relationship in leaves may integrate effects of both individual leaf and whole plant ontogenies.

Are these relationships sufficiently consistent to allow a general model to accurately predict leaf age from spectra across sites and various canopy environments?

In general, we find that the answer to this question is yes, because leaf traits (co-varying with leaf spectra) are evidently constrained by ontogenetic physiology and canopy environments. Therefore, leaf traits and spectra vary systematically and predictably with leaf age between forest sites thousands of kilometers apart and across canopy environments. This result emerges from two key findings:

(**1**) A single model, developed to predict leaf age from the spectra of sunlit leaves in a southwestern Amazon forest in Peru, predicts sunlit and shade canopy leaf ages from a central eastern Amazon forest in Brazil almost as well without recalibration. The success of the model of Chavana-Bryant *et al.* (2016) in predicting ages of sunlit and shade canopy leaves across widely separated sites (Fig. 4a,b) suggests that general rules constrain ontogenic development within similar leaf growth environments. This is also true across all tropical leaves (see finding 2, below) once the differences between canopy environments and associated environment-trait linkages are accounted for (Figs 2, 3). The underlying reason, as discussed before, is that key spectral regions were consistently associated with leaf age (Fig. 3a) and with canopy environments (Fig. 3b).

(**2**) Because leaf traits (and hence spectra) vary substantially with leaf growth environments, a more general model to accurately predict leaf ages across environments (including both canopy and subcanopy trees) can be developed (e.g. by incorporating the samples of wider trait ranges). Leaf ages predicted by the Peru model deviated from observed ages in a way that systematically and predictably depended on canopy environments, developmental pathways and leaf traits (Figs 5–8). In general, understory leaves exhibited trait values that fell outside the range exhibited by both the Peruvian and Brazilian canopy leaves. Old-leaf traits differed for understory leaves (Fig. 6), and young leaves of some

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middle-canopy and understory leaves followed a different developmental pathway manifesting reddish color in leaves early in development (Fig. 5). This distinct developmental pathway is possibly a consequence of strategies for herbivore defense in subcanopy tropical leaves based on delay of chlorophyll infusion in herbivore-abundant environments (Fig. 5a) (Kursar & Coley, 1992; Dominy *et al.*, 2002; Queenborough *et al.*, 2013).

These observations support the ideas of (a) a model that explicitly included canopy environments and leaf traits as new predictor variables alongside leaf spectra (Fig. 9), and (b) a model driven only by leaf spectra, but parameterized by leaves that span the entire trait range found in diverse canopy environments (Fig. 9).

Both of these approaches significantly improved model generality, but the spectra-only model, parameterized by leaves across all canopy environments, performed better than one fit to the sun-specific subset of traits. This confirms our hypothesis (H2 from earlier subsection 'Generalizing the leaf age model across canopy environments') that because spectra are jointly influenced by all leaf traits, whether measured or unmeasured, and therefore this spectral model had more predictive power because it could implicitly account for the effects of unmeasured traits.

We highlight three directions for further work on general leaf-age modeling. First, we note that despite the relatively strong predictive capacity of our recommended 'All Spectra' leaf age model (Fig. 9d, $R^2 \approx 0.8$ or better), there is still systematic residual variation between predictions of the spectra–age model and observations of leaf age. The residuals of the spectra–age model (Fig. S7) showed a concave nonlinear relationship with observed leaf age, with both young and old leaf ages being underestimated. This pattern, evident in both Brazil and Peru datasets (Fig. S7), suggests that even better models of leaf age may be possible with further work that identifies the causes of this residual variation, and/or through the inclusion of additional leaf variation to expand the range of the modeling approach.

Second, our demonstration here of convergent relationships across the broad trait variability induced by both leaf development and canopy environments across two distinct forests suggests that even leaf samples from a small set of individuals, if designed to encompass this breadth, may provide a powerful tool with which to predict leaf developmental trajectories and ages across additional tropical forests, and even forest systems in other biomes. Future studies could use multiple sites, biomes and plant types to investigate the feasibility of developing general, globally applicable algorithms for leaf age.

Finally, leaf traits and associated spectra evolve with development, but the similar developmental stages may be reached at different ages depending on individuals, canopy environments, and biomes. This suggests that leaves with varying lifespans should be adjusted to a common developmental trajectory (as in Chavana-Bryant *et al.*, 2016) in work seeking to generalize models of leaf age to accommodate for leaves with different lifespans. For canopy leaves in Peru vs Brazil, the difference between development-adjusted age (used for Peru leaves by Chavana-Bryant *et al.*, 2016) and absolute age (used here for Brazil leaves) was not large, as indicated by the comparably good age model fits for Peru and Brazil canopy leaves (Figs 4, 9). However, middle-canopy and understory leaves can have differences in early developmental pathways (as we have shown for reddish leaves in this study) and can have lifespans several folds longer than canopy leaves (Reich *et al.*, 2004). This implies that for a given absolute age, understory leaves are at an earlier developmental stage, leading to an underestimation of their predicted ages and a decrease in overall model performance when leaves at different developmental stages have the same absolute age. This is evident from the steeper slopes of observed vs predicted age for understory environments compared to other environments (Fig. 9). We therefore expect that modeling of middle-canopy and understory leaf age would be improved by extending observations of these leaves throughout their life cycle, until senescence (which can take up to several years). We hypothesize that in general, adjusting leaves with varying lifespans to a common developmental trajectory would reveal local (within canopy) to inter-biome convergence in relative leaf aging processes.

Conclusion

Our results show the convergent correlations among leaf traits, spectra and age across various tree species, sites and canopy environments. These results support the development of a general spectra–age model and we have shown that this model can effectively predict leaf age across the observed ontogenic and environmental variation. This study has three important implications for the broader plant science and remote sensing communities.

First, leaf spectra can allow rapid and effective estimation of leaf ages across tropical forests and various canopy environments. Our work, building on previous studies of spectral-leaf traits correlations (Asner *et al.*, 2012, 2014; Serbin *et al.*, 2012, 2014) and age-dependence (Chavana-Bryant *et al.*, 2016), shows that reconstructing life cycles of multiple physiochemical properties of leaves across forest sites and canopy environments is possible. Future spectrally derived studies should give insights into the fundamental mechanisms that regulate the life cycle of resource investments and return in leaves.

Second, the convergent spectra–age correlation suggests that remotely sensed observations using imaging spectroscopy (also known as 'hyperspectral') data could enable the monitoring and mapping of leaf age compositions across tree crowns and whole landscapes, and provide insights into temporal dynamics of leaf age demography in forest canopies. The generality of these correlations across sites and canopy environments also implies that leaf age dependencies of commonly used vegetation indices (VIs) seen at the Peru site (Chavana-Bryant *et al.*, 2016), likely affect these VIs across broad regions. Therefore, remote sensing-based studies of tropical forest seasonality and phenology should account for leaf age effects.

Finally, these findings have important theoretical implications. Leaf traits have been observed to vary substantially over their life cycles, exhibiting as much or more within-species variation than between-species variation in both temperate (McKown *et al.*, 2013; Fajardo & Siefert, 2016) and tropical (Chavana-Bryant *et al.*, 2016) trees. Our extension of this observation across sites

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and canopy environments suggests that fundamental evolutionary rules constrain the co-variations among spectra, traits and age both within and between species, and that studies that seek insights into these rules (e.g. via analysis of leaf economics; Wright *et al.*, 2004; Osnas *et al.*, 2013) should be expanded from their traditional focus on species (generally collected at peak season) to include various leaf developmental stages and their effects on key physiological traits.

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Author contributions

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., with contributions from C.C-B., S.P.S., F.G., Y.M., K.G., X.Y., L.P.A. and W.J.D.vL.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Leaf age monitoring at the Brazil site was carried out by using metal tag and *in situ* photo-documentation.

Fig. S2 Within-canopy leaf light environment (approximated by gap fraction from *in situ* hemispherical photos) is correlated with branch height above the ground and branch depth below the local canopy top.

Fig. S3 Relationships between mean leaf traits of mature leaves of each tree–environment combination and their associated canopy environments (branch height and branch depth) at the Brazil site.

Fig. S4 Flow-chart for spectra–trait analysis by using partial least-squares regression (PLSR).

Fig. S5 Mean leaf level hyperspectral reflectance for three leaf age classes (young, mature, and old) of each tree–environment combination at the Brazil site.

Fig. S6 Metrics of fit for PLSR-based leaf age models vs number of latent variables included in the models.

Fig. S7 Relationships between modeled leaf age residuals and observed leaf age.

Table S1 Summary statistics for leaf traits (LMA and LWC) and NIR reflectance (800 nm) for four canopy environments in Brazil and for two-site comparison (Brazil and Peru)

Table S2 Leaf age model performance assessment for Brazil data using three statistical metrics (R^2, RMSE and AIC)

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