

Article (refereed) - postprint

Wu, Jin; Chavana-Bryant, Cecilia; Prohaska, Neill; Serbin, Shawn P.; Guan, Kaiyu; Albert, Loren P.; Yang, Xi; van Leeuwen, Willem J.D.; Garnello, Anthony John; Martins, Giordane; Malhi, Yadvinder; Gerard, France; Oliveira, Raimundo Cosme; Saleska, Scott R. 2017. **Convergence in relationships between leaf traits, spectra and age across diverse canopy environments and two contrasting tropical forests** [in special issue: Featured papers on 'Tropical plants and ecosystem function'] *New Phytologist*, 214 (3). 1033-1048. [10.1111/nph.14051](https://doi.org/10.1111/nph.14051)

© 2016 The Authors. *New Phytologist* © 2016 New Phytologist Trust

This version available <http://nora.nerc.ac.uk/511756/>

NERC has developed NORA to enable users to access research outputs wholly or partially funded by NERC. Copyright and other rights for material on this site are retained by the rights owners. Users should read the terms and conditions of use of this material at

<http://nora.nerc.ac.uk/policies.html#access>

This document is the author's final manuscript version of the journal article, incorporating any revisions agreed during the peer review process. There may be differences between this and the publisher's version. You are advised to consult the publisher's version if you wish to cite from this article.

The definitive version is available at <http://onlinelibrary.wiley.com/>

Contact CEH NORA team at
noraceh@ceh.ac.uk

1 **Convergence in relations among leaf traits, spectra and age across diverse canopy**
2 **environments and two contrasting tropical forests**

3
4 **Authors**

5 Jin Wu^{1*}, Cecilia Chavana-Bryant², Neill Prohaska¹, Shawn P. Serbin³, Kaiyu Guan⁴, Loren P.
6 Albert¹, Xi Yang⁵, Willem J.D. van Leeuwen⁶, Anthony John Garnello¹, Giordane Martins⁷,
7 Yadvinder Malhi², France Gerard⁸, Raimundo Cosme Oliviera⁹, and Scott R. Saleska^{1*}

8
9 **Institute or laboratory of origin**

10 [1] Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721

11 [2] Environmental Change Institute, School of Geography and the Environment, University of
12 Oxford, UK

13 [3] Biological, Environmental & Climate Sciences Department, Brookhaven National Lab,
14 Upton, New York, NY 11973

15 [4] Department of Natural Resources and Environmental Sciences, University of Illinois at
16 Urbana Champaign, Urbana, IL 61801

17 [5] Department of Earth, Environmental, and Planetary Sciences, Brown University, Providence,
18 RI 02912

19 [6] School of Natural Resources and the Environment, University of Arizona, Tucson, AZ 85721

20 [7] Brazil's National Institute for Amazon Research (INPA), Manaus, Amazonas, Brazil

21 [8] Centre for Ecology and Hydrology (CEH), Wallingford, Oxfordshire OX10 8BB, UK

22 [9] Embrapa Amazônia Oriental, Pará, Brazil

23

24 ***Corresponding author**

25 Tel. +1 (520) 704-5358; Fax +1 (520) 621-9190; jinwu@email.arizona.edu (J.W.),
26 saleska@email.arizona.edu (S.R.S)

27

28 **Keywords:** Spectroscopy, partial least squares regression (PLSR), vertical canopy profiles, leaf
29 mass per area (LMA), leaf water content (LWC), vegetation indices, understory

30

31 **Type of Paper:** Original Research

32

33 **Word Count:**

34 Summary: 201 words

35 Main-Text--

36 Introduction: 1002 words

37 Methods: 2352 words

38 Results: 1681 words

39 Discussion/Conclusion: 1614+375 words

40 Acknowledgement: 118 words

41 9 Figures 1 Table

42 Supplementary materials: 7 Figures 2 Table

43

44

45 **A submission for the Special Issue “*Plant traits and ecosystem function along tropical environmental***
46 ***gradients: from leaves to landscapes*”**

47

48 **Summary**

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

68

69 **1. Introduction:**

70 It has long been recognized that many important ecological processes vary with leaf age, the
71 time elapsed since leaf budburst. During their lifetime, leaves exhibit variable photosynthetic
72 rates (Field, 1983; Reich *et al.*, 1991; Wilson *et al.*, 2001; Kitajima *et al.*, 2002; Pantin *et al.*,
73 2012), morphological changes (Maksymowych, 1973), allocation and transformation of
74 chemicals (Wilson *et al.*, 2001; Kitajima *et al.*, 2002; Pantin *et al.*, 2012), epiphyll colonization
75 (Roberts *et al.*, 1998; Toomey *et al.*, 2009), and defense against herbivory (Coley, 1980; Coley
76 & Barone, 1996; Lawrence *et al.*, 2003; Wang *et al.*, 2012). Thus, leaf age is a critical parameter
77 for interpreting leaf function over time and for understanding how leaf traits evolve over

78 development. Furthermore, expected maximum leaf age (leaf lifespan) is central to
79 understanding plant life history (Field & Mooney, 1983; Reich *et al.*, 1992), population
80 dynamics (Reich *et al.*, 2004) and the evolutionary trade-offs of the leaf economic spectrum
81 (Reich *et al.*, 1997; Wright *et al.*, 2004; Funk & Cornwell, 2013; Osnas *et al.*, 2013). Thus, many
82 disciplines have long been interested in monitoring leaf age for individual plants (Field, 1983;
83 Roberts *et al.*, 1998; Wilson *et al.*, 2001; Reich *et al.*, 2004) and leaf lifespan for many species
84 (Reich *et al.*, 1991, 1992; Wright *et al.*, 2004; Funk & Cornwell, 2013; Osnas *et al.*, 2013).

85 More recent studies have begun to emphasize the importance of leaf ages and canopy age
86 composition on phenology and ecosystem seasonality of vegetation photosynthesis and
87 transpiration (Doughty & Goulden, 2008; Richardson *et al.*, 2012; Restrepo-Coupe *et al.*, 2013;
88 Wu *et al.*, 2016). Yet leaf development is difficult to monitor at large scales, especially in carbon
89 rich tropical evergreen forests, where individual leaf ages are not as tightly synchronized with
90 phenology and ecosystem seasonality as in temperate forests (Reich, 1995). In tropical forests,
91 contrasting interpretation of satellite-detected seasonality of vegetation greenness (Morton *et al.*,
92 2014; Bi *et al.*, 2015; Saleska *et al.*, 2016) arises, in part, due to differing assumptions about the
93 distribution of leaf ages in forest canopies and how changes in age composition might affect
94 ecosystem seasonality (Doughty & Goulden, 2008; Brando *et al.*, 2010; Morton *et al.*, 2014).
95 Therefore, for such forests, ‘ground truth’ studies of seasonal leaf age dynamics are clearly
96 needed.

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

107 Spectroscopy may provide a fast and efficient means for estimating leaf ages from their
108 optical properties. Differences in the reflectance, absorbance, and transmittance of light at

109 different wavelengths by plant parts are tightly coupled to their chemical composition, cell
110 structure, and physiological properties (Curran, 1989; Elvidge, 1990; Kokaly *et al.*, 2009),
111 leading to the rapid recent development of spectroscopic methods as a general tool in plant
112 ecophysiology and ecology. For example, spectroscopy has been used to estimate wood density
113 and hydraulic traits (Acuna & Murphy, 2006; Petisco *et al.*, 2006; Luss *et al.*, 2015), accurately
114 identify plant species from dried leaves (Durgante *et al.*, 2013) across developmental stages
115 (Lang *et al.*, 2015), quantify non-structural carbohydrate content of different plant organs
116 (Ramirez *et al.*, 2015), and characterize a broad suite of leaf biophysical traits (Clark *et al.*, 2005;
117 Asner & Martin, 2011; Asner *et al.*, 2014; Serbin *et al.*, 2012, 2014).

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

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

- 133 1. How are leaf traits and spectra related with leaf development across sites and canopy
134 environments?
- 135 2. Are these relationships sufficiently consistent to allow a general model to accurately
136 predict leaf age from spectra across sites and various canopy environments?

137 To address these questions, we used measurements of reflectance spectra, traits, and age of
138 leaves collected at two tropical evergreen forests: we built upon the spectra-age model presented
139 in Chavana-Bryant *et al* (2016) that was based on sunlit leaves of a Peruvian Amazonian forest

140 and evaluated this model at an independent Brazilian site with contrasting soil and forest
141 properties. We then explored the consistency of relationships across both sites, with a view to
142 developing and validating a spectra-age model generally applicable for tropical forest leaves
143 across forest sites and canopy environments.

144

145 **2. Materials and Methods**

146 2.1 Study Sites

147 The study focuses on two Amazonian evergreen forests (Fig. 1): a Brazilian site and a
148 Peruvian site that represent contrasting edaphic and forest properties along the primary axis of
149 ecological variation across Amazonian forests. The Brazil site is less productive, higher wood
150 density and slower turnover but higher biomass forest than the Peru site (Malhi *et al.*, 2002, 2006;
151 Patino *et al.*, 2009). The contrast appears driven by soil properties, with western Amazonian
152 soils in Peru being more fertile but with poorer physical structure (Quesada *et al.*, 2012).

153 The Brazil site (2°51' S, 54°58' W) encompasses the km67 eddy flux tower and associated
154 biometric plots in Tapajos National forest, near Santarém, Brazil (Rice *et al.*, 2004; Hutyra *et al.*,
155 2007). Part of the Brazilian Large Scale Biosphere-Atmosphere Experiment in Amazonia (LBA)
156 (Davidson *et al.*, 2012), this site sits on a well-drained clay-soil plateau. Mean annual
157 precipitation is ~2000 mm/year with a 5-month-long dry season (Restrepo-Coupe *et al.*, 2013).

158 The Peru site encompasses two primary forest plots within the Tambopata National Reserve
159 in the Madre de Dios region of Peru (Malhi *et al.*, 2014), both part of the Global Ecosystems
160 Monitoring (GEM) network and the RAINFOR Amazon Forest Inventory Network (Malhi *et al.*,
161 2002), with RAINFOR codes TAM-06 (12°84' S, 69°30' W) and TAM-09 (12°83' S, 69°27' W).
162 These forests grow on Haplic alisol soils (Quesada *et al.*, 2010), at elevations of 215m and 220m
163 above sea level, respectively. Mean annual precipitation is ~1900 mm/year (Malhi *et al.*, 2014),
164 with a 4-5 month-long dry season (precipitation <100 mm/mo; Lewis *et al.*, 2011).

165

166 2.2 Field measurements

167 2.2.1 Brazil dataset

168 In campaigns conducted in August-September 2013, November 2013, March 2014, and July-
169 August 2014, we selected a subset of 11 trees (Table 1) for precise leaf age monitoring. The age
170 monitoring began with observations of leaf budburst and subsequent leaf tagging (using metal

171 tags alongside *in-situ* photos; Fig. S1) during the August-September 2013 campaign, when most
172 sampled trees were flushing new leaves. Following the initial intensive tagging work, we
173 continued to tag and photograph new leaves periodically. This age tagging technique enabled us
174 to accurately track leaf age in terms of days from leaf emergence at budburst (0 days) to old age
175 (~400 days). Aside from some of the canopy leaves, this age was not sufficient to sample the
176 senescent leaf age class.

177 We sampled a total of 759 leaves with precise leaf age information for these 11 trees,
178 consisting of 4 canopy, 3 mid-canopy, and 4 understory trees. Since we harvested both sunlit and
179 shaded leaves for canopy trees, our dataset of precise leaf age measurements is composed of 15
180 tree-environment combinations: leaves sampled from 4 canopy trees in a sunlit environment, 4
181 canopy trees in a shaded environment, 3 mid-canopy trees environment, and 4 understory trees
182 environment (Table 1).

183 We measured reflectance spectra (section 2.2.3) for all 759 leaves and leaf traits (Leaf Mass
184 per Area, LM, and Leaf Water Content, LWC) for a subset of 507 of these leaves which were
185 used for the trait-age analysis reported here. Traits were derived from fresh leaf weight
186 (precision at 0.001 g), area (using a Canon LiDE 120 scanner) and dry weight oven-dried at 60
187 °C for 72 hours.

188 We recorded leaf growth environments, including (1) *in-situ* digital hemispherical photos
189 (collected with a 180° fisheye lens adapter for a Canon T3) to capture the radiation regime
190 (section 2.2.5), (2) branch height (m)—the height of sampled leaves aboveground, and (3)
191 branch depth (m)—the depth of sampled leaves below local canopy top.

192 In addition to the 11 trees with precisely measured leaf ages, we sampled an additional 29
193 tree species across diverse canopy environments, including 7 canopy trees (crowns exposed to
194 direct sun), 10 mid-canopy trees (20-30m tall), 4 understory trees (10-20m tall), and 8 forest-
195 floor shrubs (<5 meters tall). The dataset included measurements of leaf traits (LMA and LWC),
196 reflectance spectra, and the canopy environments (i.e. vertical canopy positions where the leaves
197 were harvested). This dataset did not include precise leaf ages, but provides baseline data on
198 community level relationships between leaf traits and canopy environments for fully expanded
199 mature leaves (Fig. S4).

200

201 2.2.2 Peru dataset

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

211 We note that this method of assigning leaf ages differed from that used in Brazil, where
212 absolute ages (based on time elapsed since tagging at emergence) were used for all leaves. This
213 difference in age assignment methods results in a 1-4 week offset in age between the datasets,
214 depending on species (Brazil tagged leaves were measured for spectra when they were
215 sufficiently large, typically at 2-5 weeks since emergence, an age that was defined as 1 week for
216 Peru leaves), and a scale difference, depending on species, for old leaves (since Peru leaves were
217 scaled to reach senescence at 13 months whereas Brazil leaf ages were tracked to about 13
218 months without scaling). As shown in the results section, this difference in dating methods did
219 not significantly limit the inter-comparability of leaf age predictions between sites.

220

221 2.2.3 Spectral measurements in Brazil

222 We measured leaf spectra using a full-range (350-2500nm) FieldSpec® Pro
223 spectroradiometer (Analytical Spectra Devices, ASD, Boulder, CO). The spectrometer had a
224 spectral sampling resolution of 1.4nm, 2.2nm and 2.3nm in the visible, NIR, and SWIR
225 wavelengths and all data were interpolated to 1nm before analysis. All measurements were
226 collected using a customized assembly attached to a plant probe with an internal calibrated light
227 source, following Chavana-Bryant *et al* (2016) protocols. The customized assembly was
228 composited by two measurement blocks: one for 99.9% reflectivity white standard (Spectralon,
229 Labsphere Inc., North Dutton, NH, USA), and the other for 3% reflectivity dark standard
230 (Odyssey III black 449/9009 Marine Grade Cover Fabric). For each leaf, reflectance spectra
231 were measured on 1-6 different parts of the leaf adaxial surface and then averaged to determine
232 the mean optical properties across all wavelengths.

233

234 2.2.4 Vegetation indices (VIs)

235 To include important aspects of leaf bio-physiological traits that are not fully covered by
 236 LMA and LWC, we calculated four commonly-used VIs, including Normalized Difference
 237 Vegetation Index (NDVI; Eqn 1; Tucker, 1979; Ustin *et al.*, 2009), Enhanced Vegetation Index 2
 238 (EVI2; Eqn 2; Jiang *et al.*, 2008), Photosynthetic Reflectance Index (PRI; Eqn 3; Gamon *et al.*,
 239 1992), and Normalized Difference Water Index (NDWI; Eqn 4; Gao, 1996).

$$240 \quad NDVI = \frac{NIR - R}{NIR + R} \quad (1)$$

$$241 \quad EVI2 = 2.5 \times \frac{NIR - R}{NIR + 2.4 \times R + 1} \quad (2)$$

$$242 \quad PRI = \frac{\rho_{531} - \rho_{570}}{\rho_{531} + \rho_{570}} \quad (3)$$

$$243 \quad NDWI = \frac{NIR - SWIR}{NIR + SWIR} \quad (4)$$

244 where NIR is the reflectance at near-infrared 800 nm band, R is the reflectance at Red 680 nm
 245 band, ρ_{531} is the reflectance at 531 nm, ρ_{570} is the reflectance at 570 nm, and SWIR is the
 246 reflectance at short-wavelength infrared 1240 nm band.

247 These VIs represent important leaf bio-physiological properties: NDVI and EVI2 are the
 248 integrated metric for the greenness and structure of leaves (Sellers *et al.*, 1992; Huete *et al.*,
 249 2002); PRI is a measure of the intrinsic quantum yield for photosynthesis (Gamon *et al.*, 1992);
 250 NDWI is an indicator of leaf water content or hydrological status (Gao, 1996). By using these
 251 VIs (together with leaf traits of LMA and LWC, and spectra), we aim for a more comprehensive
 252 understanding of canopy environments effect on leaf properties and their developmental
 253 trajectories.

254

255 2.2.5 Within-canopy light environment

256 We estimated the within-canopy light environment from *in-situ* digital hemispheric photos
 257 (section 2.2.2). These photos were preprocessed and quality controlled using Adobe Lightroom 4
 258 (Adobe Systems INC., San Jose, CA). Contrast was then optimized, and the modified images
 259 were exported in JPEG format. Using a custom MATLAB program together with Otsu's

260 algorithm (Ostu, 1975), these images were automatically binarized into sky (or gap) or non-sky
261 pixels. The image fraction of the sky, or gap fraction, was then calculated to index the light
262 environment of the leaf sample.

263

264 2.3 Spectra-Age Modelling

265 2.3.1 General approach

266 As in Chavana-Bryant *et al* (2016), we used the partial least-square regression (PLSR)
267 modeling approach (Geladi & Kowalski, 1986; Wold *et al.*, 2001), which was adapted from
268 several recent studies (Wolter *et al.*, 2008; Serbin *et al.*, 2014; Singh *et al.*, 2015). PLSR is the
269 current state-of-the-art approach for linking leaf and canopy spectroscopy with leaf and plant
270 traits (e.g. Bolster *et al.*, 1996; Townsend *et al.*, 2003; Asner and Martin, 2011; Serbin *et al.*,
271 2014). Previous studies have also shown that PLSR is a more robust method compared to simple
272 correlation or multiple linear regression approaches (Geladi & Kowalski, 1986; Grossman *et al.*,
273 1996; Wold *et al.*, 2001).

274 Here the PLSR included five steps (Fig. S2): (1) filtering of outliers (which removed ~5% of
275 data) following the Monte-Carlo sampling method for outlier detection (Xu & Liang, 2001); (2)
276 the filtered dataset was one-time randomly divided into the training (70%) and testing (30%)
277 datasets; (3) 90% of the training dataset were randomly selected (with 100-time replication) for
278 PLSR analysis, with the latent variable number varying from 1 to n (n=20 in our case); (4) the
279 PLSR regression coefficients were applied to the training and testing datasets, with model
280 performance assed by using root mean squares error (RMSE), and R^2 (the proportion of variance
281 of observation explained by model); (5) the optimal latent variable number were then identified
282 by minimizing RMSE and maximizing R^2 .

283 We implemented the above PLSR analysis to our predictor variables, using the MCS function
284 from LibPLS (<http://www.libpls.net>) for outlier removal, PLSREGRESS function in Matlab
285 (Mathworks, Natick, MA, USA) for the PLSR analysis, and custom Matlab functions for other
286 steps. The predictor variables in this study can be either leaf spectra only (400–2500 nm; see
287 “Peru Spectra model” in section 2.3.2 and “All Spectra model” in section 2.3.3 below) or leaf
288 spectra combined with leaf traits (see “Peru Spectra+all Trait model” and “All Spectra+all Trait
289 model” in section 2.3.3 below).

290

291 2.3.2 Cross-site spectra-age analysis

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

300

301 2.3.3 Generalizing the leaf age model across canopy environments

302 Since leaf growth environments affect within-canopy leaf trait variation (Ellsworth & Reich,
303 1993; Cavaleri *et al.*, 2010), we expected that the leaves from the broader range of growth
304 environments in Brazil would have different optical properties (and therefore different
305 relationships between leaf spectra and age) as compared with the sunlit leaves from Peru
306 (Chavanna-Bryant *et al.*, 2016). To investigate how spectra-age relationships depend on the
307 different growth environments and their associated traits, and hence, to develop a more general
308 model of leaf age applicable across these growth environments, we first conducted a reference
309 test (“Test 0”) of how well the original Peru Spectra model predicted leaves across different sites
310 and environments. We then tested three models of leaf age--trait--spectra relations across canopy
311 environments:

- 312 - Test 1 (Peru Spectra+ all Trait model) —determines whether accounting for changing
313 growth environments and their associated leaf traits could improve performance of the
314 Peru reference model when applied to leaves from all environments. To this end, we used
315 the original leaf spectral variables for the Peru reference model alongside the added
316 variables, including branch height, depth, LMA, LWC, and four VIs, as the new predictor
317 variables, to generate a “Peru Spectra+all Trait” PLSR model.
- 318 - Test 2 (All Spectra model) —tests whether a more general spectra-only model can predict
319 leaf ages across all categories of leaves and growth environments. In this test, we used
320 combined Peru and Brazil datasets (with leaves encompassing the full range of traits that

321 emerge from development under different environments) to re-parameterize an “All
322 Spectra” model (with no traits included explicitly).

323 - Test 3 (All Spectra+all Trait model) is a simple combination of test 1 and 2.

324 We hypothesized (**H1**) that including traits and proxies for growth environments (test 1)
325 would indeed improve model generality from sunlit leaves to understory leaves. A positive
326 outcome for test 1 (which would show that accounting for environmental influence on leaf trait
327 variation improves ability to predict age) would suggest a second hypothesis (**H2**) that a spectra-
328 only model should be able to perform as well as, or even better than, the hybrid model of Test 1.
329 This is because spectral models have been shown to predict a broad array of traits (e.g. Serbin *et*
330 *al.*, 2014), including traits that are unmeasured for the leaves used in this study but which may
331 also be associated with age (Chavana-Bryant *et al.*, 2016). Finally, we hypothesize (**H3**) that test
332 3 will perform only marginally better than test 2, because the spectra will themselves already
333 capture the majority of the variation in the response as compared to including traits separately in
334 the model.

335 To test these hypotheses, in addition to the two metrics of model goodness, RMSE and R^2
336 (section 2.3.1 above), we also calculated the Akaike Information Criterion (AIC) for the model
337 cross-comparisons. The AIC used is formatted as $AIC = N \times \log(\delta^2) + 2 \times m$, following the
338 literature (Akaike, 1974; Aho *et al.*, 2014), where N is the number of leaves, δ is RMSE, and m
339 is the optimal latent variable number for each PLSR modeling scenario (section 2.3.1 above).

340

341 3. Results

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

344

345 3.1 Leaf traits and spectra vary with age across canopy environments and forests

346 Despite the broad trait variation induced by different canopy environments (from full sun to
347 deeply shaded understory environment, Table S1), correlations of leaf traits LMA and LWC with
348 leaf age were evident across the Brazilian site community ($R^2=0.20$, $p<10^{-5}$ for LMA; $R^2=0.42$,
349 $p<10^{-5}$ for LWC; Fig. 2a,b). The trait differences across canopy environments tended to obscure
350 the strength of these correlations, which were more evident within specific environments across
351 all trees ($R^2=0.23$ to 0.72 for LMA and $R^2=0.60$ to 0.80 for LWC, Fig. 2) and within

352 environments of individual trees. Within individual trees, all 11 tree-environment combinations
353 showed a significantly positive trend in LMA-age relationships ($R^2=0.52-0.91$), and significantly
354 negative LWC-age relationships ($R^2=0.60-0.95$). These positive LMA-age and negative LWC-
355 age relationships are consistent with those that were observed at the contrasting forest in Peru
356 where a single leaf environment was sampled (sunlit leaves, Chavana-Bryant *et al.*, 2006).

357 Spectral data also showed strong dependency on leaf age and leaf canopy environment across
358 all Brazilian tree-environment combinations (Fig. 3 and Fig. S7). Mean visible reflectance,
359 especially the green peak (~550 nm), and its variance showed continuous declines with age
360 across all Brazilian tree-environment combinations. Initial mean NIR reflectance (800-1200 nm)
361 increased (with lower variance) during leaf expansion, which was followed by decreases in the
362 mean (and increases in variance) as leaves aged (Fig. 3a,c). Mean SWIR reflectance (1400-2500
363 nm) increased monotonically with age, while the variance initially decreased and then increased
364 as leaves aged. These patterns of the relative spectra change with leaf age, observed at the Brazil
365 site across vertical canopy profiles, are also consistent with those observed in the Peruvian sunlit
366 leaves (Chavana-Bryant *et al.*, 2016).

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

373

374 3.2 Cross-site spectra-age analysis

375 Since leaf traits and spectra consistently vary with leaf age across both canopy environments
376 and forest sites (albeit with offsets among the different canopy environments), we explored the
377 application of the Peru-trained spectra-age model to the independent forest in Brazil. We found
378 that the Peruvian model developed in Chavana-Bryant *et al* (2016, with 7 latent variables)
379 predicted ages of leaves for the Brazilian canopy trees with high precision ($R^2=0.75$ and $R^2=0.78$
380 for sunlit and shade canopy leaves respectively) (see Fig 4a,b at 7 latent variables, and Fig. 9a).
381 However, model performance when predicting ages of leaves from middle and understory

382 Brazilian trees was poor ($R^2=0.27$ and $R^2=0.29$ for Brazilian middle-canopy and understory
383 leaves respectively) (Fig 4a,b for 7 latent variables; Fig. 9a).

384 We sequentially re-fitted the Peru-trained model with different numbers of latent variables,
385 in order to investigate whether there existed an optimum number of latent variables that would
386 improve the Peru-trained model performance across the range of canopy environments at the
387 Brazil site. We found that reducing the number of latent variables from 7 to 5 significantly
388 improved performance of the Peru-trained model in predicting the ages of Brazil middle-canopy
389 and understory leaves, without resulting in a significant reduction in performance for Peruvian
390 sunlit leaves (for which the 7-variable model was optimal) (Fig. 4a,b). We thus adopted the 5-
391 variable model as the Peru reference model, optimized across canopy environments.

392 Closer investigation of the performance of the 7-variable Peru-trained spectra-age model for
393 individual Brazilian trees revealed that the relatively poor performance of this model relative to
394 the 5-variable model was confined to early developing leaves (≤ 40 days old) of one middle-
395 canopy and two understory trees (*E.uchi_MC*, *G.Amazonicum_US*, and *M.ruficalyx_US*; Table 1
396 and Fig. 5). The early developing leaves of these trees exhibited “reddish” coloration (e.g. Fig.
397 5a), a common early developmental process displayed by sub-canopy leaves but not by canopy
398 sunlit leaves, which has confounding effects for the 7-variable Peru model (Fig. 5c).

399 The PLSR regression coefficients (Fig. 4c) and Variable Importance in Projection (VIP; Fig.
400 4d) from this optimized reference model indicated the important spectral domains responsible for
401 leaf age modeling, which included visible domain (especially ~ 550 nm), red edge (~ 725 nm),
402 NIR (~ 800 nm) and several water absorption bands (~ 1440 nm, ~ 1700 nm, and ~ 1920 nm).
403 These patterns also matched well with the age-dependent spectral variation (Fig. 3a,c), providing
404 confidence for our spectra-age modeling.

405 The optimized Peru model performance using the Peru testing data was $R^2=0.83$ and
406 RMSE=55 days compared with $R^2=0.64$ and RMSE=72 days when applied to all Brazilian data
407 (Fig. 9b). When the Brazil data were separated by canopy environments, cross-site performance
408 of the Peru model applied to the Brazil data ranged from high performance in sunlit and shade
409 canopy leaves and middle-canopy leaves ($R^2=0.77$, RMSE=62 days for sunlit, and $R^2=0.77$,
410 RMSE=62 days for shade canopy leaves, and $R^2=0.71$, RMSE=80 days for middle-canopy leaves)
411 to lower performance when applied to understory leaves ($R^2=0.29$, RMSE=101 days for the
412 original Peru model and $R^2=0.47$, RMSE=90 days for the optimized Peru model) (Fig. 9a,b). In

413 sum, we found that the greater the disparity in canopy environment from that used to train the
414 Peru reference model, the larger the inaccuracies in leaf age model predictions.

415

416 3.3 The range of leaf traits affects cross-site model generality

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

429 To leverage this result, we quantified how model fit of the optimized Peru reference model
430 (developed for Peruvian sunlit leaves) depended on different environments. Deviations of
431 predictions from observations were characterized by simple linear regressions for each tree-
432 environment combination (as seen by regression lines between model predicted and observed
433 ages, Fig. 7). These regression lines showed systematic deviation from the 1:1 line, with strong
434 dependency on leaf growth environments: Deviations in canopy sunlit environments (Fig. 7a-d)
435 usually (3 of 4 trees) followed a slope shallower than the 1:1 line, while at the other end of the
436 environmental gradient, deviations in Brazilian understory trees were significantly steeper than
437 the 1:1 line (Fig. 7l-o). Deviations were largest in understory environments, whose leaf ages
438 tended to be significantly overestimated in young age classes, but underestimated in old age
439 classes.

440 We found that the deviation of leaf age model performance could be tied to canopy
441 environments (and to leaf traits): the deviations, as represented by the variation in the parameters
442 (slope and intercept) of lines fit to those deviations, were systematically explained by growth
443 environments, as captured by branch height (Fig. 8a,c), and by traits (e.g. LMA, Fig. 8b,d).

444 Therefore, variability in canopy environments and traits is the source of the lower performance
445 of the optimized Peru leaf age model when extended to new canopy environments. This suggests
446 that modeling strategies that account for variation in traits or proxies for canopy environment
447 (e.g. branch height) should produce more general models of leaf age that are applicable across
448 different canopy growth environments.

449

450 3.4 Generalizing the leaf age model across canopy environments

451 Four modeling exercises were conducted to explore model generality across canopy
452 environments in Brazil (Fig. 9), with the optimum five latent variables for “Peru Spectra” and
453 “All Spectra” models and the optimum six latent variables for “Peru Spectra+all Trait” and “All
454 Spectra+all Trait” models (Fig. S6). The calculated AIC metric for each modeling scenario
455 showed consistent, positive relationship with RMSE, suggesting that RMSE is a good metric for
456 the cross-model comparison in this study (Table S2). Relative to the Peru reference model (Fig.
457 9a), the “Peru Spectra+all Trait” model, which incorporated the covariates of growth
458 environments and leaf traits, modestly improved overall performance for all the Brazil data
459 ($R^2=0.69$, RMSE=74 days vs $R^2=0.64$, RMSE=72 days for reference; Fig. 9c), but significantly
460 improved prediction for Brazilian understory samples (R^2 increased from 0.47 to 0.57; RMSE
461 decreased from 90 days to 88 days).

462 The “All Spectra” model, parameterized by both Peruvian and Brazilian leaf spectra (Fig. 9d),
463 achieved large performance gains across canopy environments (R^2 increased from 0.64 to 0.79
464 and RMSE decreased from 72 days to 53 days for all Brazilian leaves; R^2 increased from 0.47 to
465 0.73 and RMSE decreased from 90 days to 72 days for Brazilian understory leaves).

466 The “All Spectra+all Trait” model, parameterized by both Peruvian and Brazilian leaf spectra
467 and traits (Fig. 9e), led to the best model overall, but, as we hypothesized (H3 from §2.3.3
468 above), it gave only a modest improvement over the “All Spectra” model overall (R^2 increased
469 from 0.79 to 0.81 and RMSE decreased from 53 days to 50 days; Fig. 9). Specifically, the most
470 significant improvement occurred in Brazilian understory leaves (R^2 increased from 0.73 to 0.82
471 and RMSE decreased from 72 days to 60 days; Fig. 9), at the small expense of model
472 performance for Brazilian canopy shade leaves (R^2 decreased from 0.89 to 0.88 and RMSE
473 increased from 48 days to 49 days; Fig. 9).

474

475 4. Discussion

476 We investigated whether principles of leaf trait ecology and ontogeny could be used to create
477 a general model relating leaf spectra to leaf age, taking into account the effect of growth
478 environments. We divided this investigation into two broad questions: (1) How are leaf traits and
479 spectra related to leaf development across different sites and canopy growth environments? (2)
480 Are these relationships sufficiently consistent to allow a general model to accurately predict leaf
481 age from spectra across sites and various canopy environments?

482

483 I. How are leaf traits and spectra related to leaf development across different sites and 484 canopy growth environments?

485 Two key findings address this question:

486 1. *Variation in leaf traits and spectra across all leaves is large compared to datasets that*
487 *focus only on sunlit mature leaves.* Our 759 leaves from 11 trees in Brazil encompassed variation
488 in LMA (35-270 g/m²), LWC (42-83%) and NIR reflectance (0.35-0.64) (Table S1) that covers
489 over 98% LMA values and 89% NIR reflectance values recorded for the much larger dataset of
490 1,449 tree species (6,136 leaves) in Asner *et al* (2011, 2014). These results are consistent with
491 those reported at the contrasting forest site with very different soil condition (Quesada *et al.*,
492 2012) in Peru (Chavana-Bryant *et al.*, 2016) and show that such large variation in traits and
493 spectra can be attributed primarily to the substantial variation across leaf ages (Figs. 2,3) and
494 canopy environments (Figs. 2,3, and S4).

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

503

504 2. *Leaf traits and spectra vary with both age and canopy environments (Figs. 2,3,6; Fig. S4):*

505 Previous studies have found that leaf age influences leaf traits and spectra (Field & Mooney,

506 1983; Kitajima *et al.*, 1997, 2002; Roberts *et al.*, 1998; Wilson *et al.*, 2001; Yang *et al.*, 2014;
 507 Chavana-Bryant *et al.*, 2016) and that canopy environments influence leaf traits (e.g.
 508 Lichtenthaler *et al.*, 1981; Givnish *et al.*, 1988; Ellsworth & Reich, 1993; Terashima *et al.*, 2001;
 509 Koike *et al.*, 2001; Kumagai *et al.*, 2001; Cavaleri *et al.*, 2010; Kenzo *et al.*, 2015; Coble &
 510 Cavaleri, 2015), but this study also finds that understanding their interaction (Fig 2, Fig 3a,b) is
 511 particularly important for developing general relationships between leaf ages and trait-mediated
 512 spectra.

513 These interacting trait-age and trait-environment relationships lay the foundation for
 514 addressing our second question (below). This is because the age-dependent and/or environment-
 515 dependent changes in the above-mentioned leaf traits and other related leaf morphological,
 516 structural and physico-chemical traits are known to influence leaf optical properties (Curran,
 517 1989; Carter *et al.*, 1989; Elvidge, 1990; Jacquemoud & Baret, 1990; Kokaly *et al.*, 2009; Asner
 518 *et al.*, 2011, 2014; Serbin *et al.*, 2012, 2014; Yang *et al.*, 2014; Chavana-Bryant *et al.*, 2016).
 519 Intriguingly, the spectrally-based species identification study of Lang *et al* (2015) noted a result
 520 that leaves from young and adult plants differed consistently in their near-infrared spectra, which
 521 parallels what we investigated here (and also reported in Chavana-Bryant *et al.*, 2016) across the
 522 developmental stages of individual leaves within adults. This suggests that, ultimately, the
 523 spectra-age relationship in leaves may integrate effects of both individual leaf and whole plant
 524 ontogenies.

525

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

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

533

534 *1. A single model, developed to predict leaf age from the spectra of sunlit leaves in a*
 535 *southwestern Amazon forest in Peru, predicts sunlit and shade canopy leaf ages from a central*
 536 *eastern Amazon forest in Brazil almost as well without recalibration.* The success of the model

537 of Chavana-Bryant *et al* (2016) in predicting ages of sunlit and shade canopy leaves across
538 widely separated sites (Fig. 4a,b) suggests that general rules constrain ontogenic development
539 within similar growth environments. This is also true across all tropical leaves (see finding 2,
540 below) once the differences between canopy environments and associated environment-trait
541 linkages are accounted for (Figs. 2,3). The underlying reason, as discussed before, is that key
542 spectral regions were consistently associated with leaf age (Fig. 3a) and with different growth
543 environments (Fig. 3b).

544

545 *2. Because leaf traits (and hence spectra) vary substantially with growth environments, a more*
546 *general model to accurately predict leaf ages across environments (including both canopy and*
547 *subcanopy trees) can be developed (e.g. by incorporating the samples of wider trait ranges).*

548 Leaf ages predicted by the Peru model deviated from observed ages in a way that
549 systematically and predictably depended on canopy growth environments, developmental
550 pathways and leaf traits (Figs. 5-8). In general, understory leaves exhibited trait values that fell
551 outside the range exhibited by both the Peruvian and Brazilian canopy leaves. Old-leaf traits
552 differed for understory leaves (Fig. 6), and young leaves of some middle-canopy and understory
553 leaves followed a different developmental pathway manifesting reddish color in leaves early in
554 development (Fig. 5). This distinct developmental pathway is possibly a consequence of
555 strategies for herbivore defense in sub-canopy tropical leaves based on delay of chlorophyll
556 infusion in herbivore-abundant environments (Fig. 5a) (Kursar & Coley, 1992; Dominy *et al.*,
557 2002; Queenborough *et al.*, 2013).

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

562 Both of these approaches significantly improved model generality, but the spectra-only
563 model, parameterized by leaves across all canopy environments, performed better than one fit to
564 the sun-specific subset of traits. This confirms our hypothesis (H2 from §2.3.3 above) that
565 because spectra are jointly influenced by all leaf traits, whether measured or unmeasured, and
566 therefore this spectral model had more predictive power because it could implicitly account for
567 the effects of unmeasured traits.

568 We highlight three directions for further work on general leaf-age modeling. First, we note
569 that despite the relatively strong predictive capacity of our recommended “all spectra” leaf age
570 model (Fig. 9c, $R^2 \approx 0.8$ or better), there is still systematic residual variation between predictions
571 of the spectra-age model and observations of leaf age. The residuals of the spectra-age model
572 (Fig. S5) showed a concave nonlinear relationship with observed leaf age, with both young and
573 old leaf ages being under-estimated. This pattern, evident in both Brazil and Peru datasets (Fig
574 S5), suggests that even better models of leaf age may be possible with further work that
575 identifies the causes of this residual variation, and/or through the inclusion of additional leaf
576 variation to expand the range of the modeling approach.

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

584 Finally, leaf traits and associated spectra evolve with development, but similarly
585 developmental stages may be reached at different ages depending on individuals, canopy
586 environments, and biomes. This suggests that leaves with varying lifespans should be adjusted to
587 a common developmental trajectory (as in Chavana-Bryant *et al.*, 2016) in work seeking to
588 generalize models of leaf age to accommodate for leaves with different lifespans. For canopy
589 leaves in Peru versus Brazil, the difference between development-adjusted age (used for Peru
590 leaves by Chavana-Bryant *et al.*, 2016) and absolute age (used here for Brazil leaves) was not
591 large, as indicated by the comparably good age model fits for Peru and Brazil canopy leaves
592 (Figs. 4,9). However, middle-canopy and understory leaves can have differences in early
593 developmental pathways (as we have shown for reddish leaves in this study) and can have
594 lifespans several folds longer than canopy leaves (Reich *et al.*, 2004). This implies that for a
595 given absolute age, understory leaves are at an earlier developmental stage, leading to an
596 underestimation of their predicted ages and a decreased in overall model performance when
597 leaves at different developmental stages have the same absolute age. This is evident from the
598 steeper slopes of observed versus predicted age for understory environments compared to other

599 environments (Fig. 9). We therefore expect that modeling of middle-canopy and understory leaf
600 age would be improved by extending observations of these leaves throughout their life cycle,
601 until senescence (which can take up to several years). We hypothesize that in general, adjusting
602 leaves with varying lifespans to a common developmental trajectory would reveal local (within
603 canopy) to inter-biome convergence in relative leaf aging processes.

604

605 **5. Conclusion**

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

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

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

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

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

636

637 **Acknowledgments**

638 Research in Brazil was supported by NSF (PIRE #0730305), NASA (#NNX11AH24G and
639 NESSF to J.W.), and DOE (GoAmazon #DE-SC0008383). We thank LBA for support, and Dan
640 Metcalfe, Ty Taylor, Kleber Campos, and Katie Berg for assistance. Research in Peru was
641 supported by NERC, via FSF equipment loans and grants to CCH-B (TROBIT project
642 #NE/D005469/1), and to FG via CEH. We thank Olivier Jaudoin, Michael Eltringham, Stefan
643 Curtis, Ana Lombardero Morán, Valentine Alt and Italo Treviño Zeballos for assistance, and
644 Eric Cosio for logistical support in Peru. SPS was supported by the Next-Generation Ecosystem
645 Experiment (NGEE-Tropics) project supported by the U.S. DOE, Office of Science, Office of
646 Biological and Environmental Research and through contract #DE-SC00112704 to Brookhaven
647 National Laboratory.

648

649 **Author contributions**

650 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.,
651 L.P.A., C.C-B, R.C.O. and G.M. designed, performed, and/or supported Brazil field data
652 collection. Data integration and quality assurance at Brazil site were performed by J.W. J.W. and
653 S.P.S designed the Matlab version of PLSR analysis with input from C.C-B. Data analysis was
654 performed by J.W. J.W. wrote the manuscript in close collaboration with S.R.S., with
655 contributions from C.C-B, S.P.S, F.G., Y.M., K.G., X.Y., L.P.A., and W.L.

656

657 **References**

- 658 **Acuna MA, Murphy GE.** 2006. Use of near infrared spectroscopy and multivariate analysis to
 659 predict wood density of Douglas-fir from chain saw chips. *Forest Prod J.*, **56**: 67-72.
- 660 **Aho K, Derryberry D, Peterson T.** 2014. Model selection for ecologists: the worldviews of
 661 AIC and BIC. *Ecology*, **95**: 631-636.
- 662 **Akaike H.** 1974. A new look at the statistical model identification. *Automatic Control, IEEE*
 663 *Transactions on*, **19**: 716-723.
- 664 **Asner GP, Martin RE.** 2011. Canopy phylogenetic, chemical and spectral assembly in a
 665 lowland Amazonian forest. *New Phytologist*, **189**: 999-1012.
- 666 **Asner GP, Martin RE, Tupayachi R, Emerson R, Martinez P, Sinca F, Powell GV, Wright**
 667 **SJ, Lugo AE.** 2011. Taxonomy and remote sensing of leaf mass per area (LMA) in
 668 humid tropical forests. *Ecological Applications*, **21**: 85-98.
- 669 **Asner GP, Martin RE, Suhaili AB.** 2012. Sources of canopy chemical and spectral diversity in
 670 lowland Bornean forest. *Ecosystems*, **15**: 504-517.
- 671 **Asner GP, Martin RE, Carranza-Jimenez L, Sinca F, Tupayachi R, Anderson CB,**
 672 **Martinez P.** 2014. Functional and biological diversity of foliar spectra in tree canopies
 673 throughout the Andes to Amazon region. *New Phytologist*, **204**: 127-139.
- 674 **Bi J, Knyazikhin Y, Choi S, Park T, Barichivich J, Ciais P, Fu R, Ganguly S, Hall F,**
 675 **Hilker T et al.** 2015. Sunlight mediated seasonality in canopy structure and
 676 photosynthetic activity of Amazonian rainforests. *Environmental Research Letters*, **10**:
 677 064014.
- 678 **Bolster KL, Martin ME, Aber JD.** 1996. Determination of carbon fraction and nitrogen
 679 concentration in tree foliage by near infrared reflectance: A comparison of statistical
 680 methods. *Canadian Journal of Forest Research*, **26**: 590-600.
- 681 **Brando PM, Goetz SJ, Baccini A, Nepstad DC, Beck PS, Christman MC.** 2010. Seasonal and
 682 interannual variability of climate and vegetation indices across the Amazon. *Proceedings*
 683 *of the National Academy of Sciences*, **107**: 14685-14690.
- 684 **Carter GA.** 1993. Responses of leaf spectral reflectance to plant stress. *American Journal of*
 685 *Botany*, 239-243.
- 686 **Cavaleri MA, Oberbauer SF, Clark DB, Clark DA, Ryan MG.** 2010. Height is more
 687 important than light in determining leaf morphology in a tropical forest. *Ecology*, **91**:
 688 1730-1739.
- 689 **Chavana-Bryant C, Malhi Y, Wu J, Asner GP, Anatsiou A, Enquist BJ, Saleska SR,**
 690 **Doughty C, Gerard F.** Leaf aging of Amazonian canopy trees revealed by spectral and
 691 physiochemical measurements. *New Phytologist*, doi: 10.1111/nph.13853
- 692 **Chen CC, Chen H, Chen YR.** 2009. A new method to measure leaf age: leaf measuring-interval
 693 index. *American journal of botany*, **96**: 1313-1318.
- 694 **Clark ML, Roberts DA, Clark DB.** 2005. Hyperspectral description of tropical rain forest tree
 695 species at leaf to crown scales. *Remote Sensing of Environment*, **96**: 375-398.
- 696 **Coble AP, Cavaleri MA.** 2015. Light acclimation optimizes leaf functional traits despite height-
 697 related constraints in a canopy shading experiment. *Oecologia*, **177**: 1131-1143.
- 698 **Coley PD.** 1980. Effects of leaf age and plant life history patterns on herbivory. *Nature*, **284**:
 699 545-546.
- 700 **Coley PD, Barone JA.** 1996. Herbivory and plant defenses in tropical forests. *Annual review of*
 701 *ecology and systematics*, 305-335.

- 702 **Curran PJ.** 1989. Remote sensing of foliar chemistry. *Remote sensing of Environment*, **30**: 271-
703 278.
- 704 **Davidson EA, de Araújo AC, Artaxo P, Balch JK, Brown IF, Bustamante MM, Coe MT,**
705 **DeFries RS, Keller M, Longo M et al.** 2012. The Amazon basin in transition.
706 *Nature*, **481**: 321-328.
- 707 **Dillen SY, de Beeck MO, Hufkens K, Buonanduci M, Phillips NG.** 2012. Seasonal patterns of
708 foliar reflectance in relation to photosynthetic capacity and color index in two co-
709 occurring tree species, *Quercus rubra* and *Betula papyrifera*. *Agricultural and forest*
710 *meteorology*, **160**: 60-68.
- 711 **Doughty CE, Goulden ML.** 2008. Seasonal patterns of tropical forest leaf area index and CO₂
712 exchange. *Journal of Geophysical Research: Biogeosciences (2005–2012)*, **113**(G1).
- 713 **Durgante FM, Higuchi N, Almeida A, Vicentini A.** 2013. Species Spectral Signature:
714 Discriminating closely related plant species in the Amazon with Near-Infrared Leaf-
715 Spectroscopy. *Forest Ecol Manag*, **209**: 240-248.
- 716 **Ellsworth DS, Reich PB.** 1993. Canopy structure and vertical patterns of photosynthesis and
717 related leaf traits in a deciduous forest. *Oecologia*, **96**: 169-178.
- 718 **Elvidge CD.** 1990. Visible and near infrared reflectance characteristics of dry plant
719 materials. *Remote Sensing*, **11**: 1775-1795.
- 720 **Erickson RO, Michelini FJ.** 1957. The plastochron index. *American Journal of Botany*, 297-
721 305.
- 722 **Fajardo A, Siefert A.** 2016. Phenological variation of leaf functional traits within species.
723 *Oecologia*: 1-9.
- 724 **Field C.** 1983. Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control
725 on the allocation program. *Oecologia*, **56**: 341-347.
- 726 **Field C, Mooney HA.** 1983. Leaf age and seasonal effects on light, water, and nitrogen use
727 efficiency in a California shrub. *Oecologia*, **56**: 348-355.
- 728 **Funk JL, Cornwell WK.** 2013. Leaf traits within communities: context may affect the mapping
729 of traits to function. *Ecology*, **94**: 1893-1897.
- 730 **Gamon JA, Penuelas J, Field CB.** 1992. A narrow-waveband spectral index that tracks diurnal
731 changes in photosynthetic efficiency. *Remote Sensing of environment*, **41**: 35-44.
- 732 **Gao BC.** 1996. NDWI—a normalized difference water index for remote sensing of vegetation
733 liquid water from space. *Remote sensing of environment*, **58**: 257-266.
- 734 **Geladi P, Kowalski BR.** 1986. Partial least-squares regression: a tutorial. *Analytica chimica*
735 *acta*, **185**: 1-17.
- 736 **Givnish TJ.** 1988. Adaptation to sun and shade: a whole-plant perspective. *Functional Plant*
737 *Biology*, **15**: 63-92.
- 738 **Grossman YL, Ustin SL, Jacquemoud S, Sanderson EW, Schmuck G, Verdebout J.** 1996.
739 Critique of stepwise multiple linear regression for the extraction of leaf biochemistry
740 information from leaf reflectance data. *Remote Sensing of Environment*, **56**: 182-193.
- 741 **Huete AR, Didan K, Miura T, Rodriguez EP, Gao X, Ferreira LG.** 2002. Overview of the
742 radiometric and biophysical performance of the MODIS vegetation indices. *Remote*
743 *Sensing of Environment*, **83**: 195-213.
- 744 **Hulshof CM, Violle C, Spasojevic MJ, McGill B, Damschen E, Harrison S, Enquist B.** 2013.
745 Intra-specific and inter-specific variation in specific leaf area reveal the importance of
746 abiotic and biotic drivers of species diversity across elevation and latitude. *Journal of*
747 *Vegetation Science*, **24**: 921-931.

- 748 **Hutyra LR, Munger JW, Saleska SR, Gottlieb E, Daube BC, Dunn AL, Amaral DF, de**
 749 **Camargo PB, Wofsy SC.** 2007. Seasonal controls on the exchange of carbon and water
 750 in an Amazonian rain forest. *Journal of Geophysical Research: Biogeosciences* (2005–
 751 2012), **112**(G3).
- 752 **Jacquemoud S, Baret F.** 1990. PROSPECT: A model of leaf optical properties spectra. *Remote*
 753 *sensing of environment*, **34**: 75-91.
- 754 **Jiang Z, Huete AR, Didan K, Miura T.** 2008. Development of a two-band enhanced
 755 vegetation index without a blue band. *Remote Sensing of Environment*, **112**: 3833-3845.
- 756 **Keenan TF, Darby B, Felts E, Sonnentag O, Friedl MA, Hufkens K, O’Keefe J, Klosterman**
 757 **S, Munger JW, Richardson AD.** 2014. Tracking forest phenology and seasonal
 758 physiology using digital repeat photography: a critical assessment. *Ecological*
 759 *Applications*, **24**: 1478-1489.
- 760 **Kenzo T, Inoue Y, Yoshimura M, Yamashita M, Tanaka-Oda A, Ichie T.** 2015. Height-
 761 related changes in leaf photosynthetic traits in diverse Bornean tropical rain forest trees.
 762 *Oecologia*, **177**: 191-202.
- 763 **Kitajima K, Mulkey SS, Wright SJ.** 1997. Decline of photosynthetic capacity with leaf age in
 764 relation to leaf longevities for five tropical canopy tree species. *American Journal of*
 765 *Botany*, **84**: 702-702.
- 766 **Kitajima K, Mulkey SS, Samaniego M, Wright SJ.** 2002. Decline of photosynthetic capacity
 767 with leaf age and position in two tropical pioneer tree species. *American Journal of*
 768 *Botany*, **89**: 1925-1932.
- 769 **Koike T, Kitao M, Maruyama Y, Mori S, Lei TT.** 2001. Leaf morphology and photosynthetic
 770 adjustments among deciduous broad-leaves trees within the vertical canopy profile. *Tree*
 771 *Physiology*, **21**: 951-958.
- 772 **Kokaly RF, Asner GP, Ollinger SV, Martin ME, Wessman CA.** 2009. Characterizing canopy
 773 biochemistry from imaging spectroscopy and its application to ecosystem studies. *Remote*
 774 *Sensing of Environment*, **113**: S78-S91.
- 775 **Kramer K, Leinonen I, Loustau D.** 2000. The importance of phenology for the evaluation of
 776 impact of climate change on growth of boreal, temperate and Mediterranean forests
 777 ecosystems: an overview. *International Journal of Biometeorology*, **44**: 67-75.
- 778 **Kumagai TO, Kuraji K, Noguchi H, Tanaka Y, Tanaka K, Suzuki M.** 2001. Vertical profiles
 779 of environmental factors within tropical rainforest, Lambir Hills National Park, Sarawak,
 780 Malaysia. *Journal of Forest Research*, **6**: 257-264.
- 781 **Kursar TA, Coley PD.** 1992. Delayed greening in tropical leaves: an antiherbivore defense?.
 782 *Biotropica*, **24**: 256-262.
- 783 **Lang C, Costa FRC, Camargo JLC, Durgante FM, Vicentini A.** 2015. Near infrared
 784 spectroscopy facilitates rapid identification of both young and mature Amazonian tree
 785 species. *PLOS One*, **10**: e0134521.
- 786 **Lawrence R, Potts BM, Whitham TG.** 2003. Relative importance of plant ontogeny, host
 787 genetic variation, and leaf age for a common herbivore. *Ecology*, **84**: 1171-1178.
- 788 **Lewis SL, Brando PM, Phillips OL, van der Heijden GM, Nepstad D.** 2011. The 2010
 789 amazon drought. *Science*, **331**: 554-554.
- 790 **Lichtenthaler HK, Buschmann C, Döll M, Fietz HJ, Bach T, Kozel U, Meier D, Rahmsdorf**
 791 **U.** 1981. Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of
 792 high-light and low-light plants and of sun and shade leaves. *Photosynthesis research*, **2**:
 793 115-141.

- 794 **Luss S, Schwanninger M, Rosner S.** 2015. Hydraulic traits of Norway spruce sapwood
795 estimated by Fourier transform near-infrared spectroscopy (FT-NIR). *Canadian Journal*
796 *of Forest Research*, **45**: 625-631.
- 797 **Maksymowych R.** 1973. *Analysis of leaf development* (No. 1). CUP Archive.
- 798 **Malhi Y, Phillips OL, Lloyd J, Baker T, Wright J, Almeida S, Arroyo L, Frederiksen T,**
799 **Grace J, Higuchi N et al.** 2002. An international network to monitor the structure,
800 composition and dynamics of Amazonian forests (RAINFOR). *Journal of Vegetation*
801 *Science*, **13**: 439-450.
- 802 **Malhi Y, Wood D, Baker TR, Wright J, Phillips OL, Cochrane T, Meir P, Chave J,**
803 **Almeida S, Arroyo L, et al.** 2006. The regional variation of aboveground live biomass in
804 old-growth Amazonian forests. *Global Change Biology*, **12**: 1107-1138.
- 805 **Malhi Y, Amézquita F, Doughty C E, Silva-Espejo JE, Girardin CA, Metcalfe DB, Aragao**
806 **LEOC, Huaraca-Quispe LP, Alzamora-Taype I, Eguluz-Mora L et al.** 2014. The
807 productivity, metabolism and carbon cycle of two lowland tropical forest plots in south-
808 western Amazonia, Peru. *Plant Ecology & Diversity*, **7**: 85-105.
- 809 **McKown AD, Guy RD, Azam MS, Drewes EC, Quamme LK.** 2013. Seasonality and
810 phenology alter functional leaf traits. *Oecologia* **172**(3): 653-665.
- 811 **Meicenheimer RD.** 2014. The plastochron index: Still useful after nearly six decades. *American*
812 *journal of botany*, **101**: 1821-1835.
- 813 **Morton DC, Nagol J, Carabajal CC, Rosette J, Palace M, Cook BD, Vermote EF, Harding**
814 **JD, North PR.** 2014. Amazon forests maintain consistent canopy structure and
815 greenness during the dry season. *Nature*, **506**: 221-224.
- 816 **Nilson T, Pisek J, Rautiainen M, Peterson U.** 2012. *Seasonal reflectance courses of forests*.
817 INTECH Open Access Publisher.
- 818 **Osnas JL, Lichstein JW, Reich PB, Pacala SW.** 2013. Global leaf trait relationships: mass,
819 area, and the leaf economics spectrum. *Science*, **340**: 741-744.
- 820 **Opler PA, Frankie GW, Baker HG.** 1980. Comparative phenological studies of treelet and
821 shrub species in tropical wet and dry forests in the lowlands of Costa Rica. *The Journal*
822 *of Ecology*, 167-188.
- 823 **Otsu N.** 1975. A threshold selection method from gray-level histograms. *Automatica*, **11**: 23-27.
- 824 **Pantin F, Simonneau T, Muller B.** 2012. Coming of leaf age: control of growth by hydraulics
825 and metabolics during leaf ontogeny. *New Phytologist*, **196**: 349-366.
- 826 **Patiño S, Lloyd J, Paiva R, Baker TR, Quesada CA, Mercado LM, Schmerler J, Schwarz**
827 **M, Santos AJB, Aguilar A, et al.** 2009. Branch xylem density variations across the
828 Amazon Basin. *Biogeosciences*, **6**: 545-568.
- 829 **Petisco C, Garcia-Criado B, Mediavilla S, de Aldana BRV, Zabalgoceazcoa I, Garcia-**
830 **Ciudad A.** 2006. Near-infrared reflectance spectroscopy as a fast and non-destructive
831 tool to predict foliar organic constituents of several woody species. *Analytical and*
832 *Bioanalytical Chemistry* **386**: 1823-1833.
- 833 **Queenborough SA, Metz MR, Valencia R, Wright SJ.** 2013. Demographic consequences of
834 chromatic leaf defence in tropical tree communities: do red young leaves increase growth
835 and survival?. *Annals of botany*, **112**: 677-684.
- 836 **Quesada CA, Lloyd J, Schwarz M, Patiño S, Baker TR, Czimczik C, Fyllas NM, Martinelli**
837 **L, Nardoto GB, Schmerler J et al.** 2010. Variations in chemical and physical properties
838 of Amazon forest soils in relation to their genesis. *Biogeosciences*, **7**: 1515-1541.

- 839 **Quesada CA, Phillips OL, Schwarz M, Czimczik CI, Baker TR, Patino S, Fyllas NM,**
840 **Hodnett MG, Herrera R, Almeida S, et al.** 2012. Basin-wide variations in Amazon
841 forest structure and function are mediated by both soils and climate. *Biogeosciences*, **9**:
842 2203-2246.
- 843 **Ramirez JA, Posada JM, Handa IT, Hoch G, Vohland M, Messier C, Reu B.** 2015. Near-
844 infrared spectroscopy (NIRS) predicts non-structural carbohydrate concentrations in
845 different tissue types of a broad range of tree species. *Methods in Ecology and Evolution*,
846 **6**, 1018-1025
- 847 **Reich PB, Walters MB, Ellsworth DS.** 1991. Leaf age and season influence the relationships
848 between leaf nitrogen, leaf mass per area and photosynthesis in maple and oak trees.
849 *Plant, Cell & Environment*, **14**: 251-259.
- 850 **Reich PB, Walters MB, Ellsworth DS.** 1992. Leaf life-span in relation to leaf, plant, and stand
851 characteristics among diverse ecosystems. *Ecological monographs*, **62**: 365-392.
- 852 **Reich PB.** 1995. Phenology of tropical forests: patterns, causes, and consequences. *Canadian*
853 *Journal of Botany*, **73**: 164-174.
- 854 **Reich PB, Walters MB, Ellsworth DS.** 1997. From tropics to tundra: global convergence in
855 plant functioning. *Proceedings of the National Academy of Sciences*, **94**: 13730-13734.
- 856 **Reich PB, Uhl C, Walters MB, Prugh L, Ellsworth DS.** 2004. Leaf demography and
857 phenology in Amazonian rain forest: a census of 40 000 leaves of 23 tree species.
858 *Ecological Monographs*, **74**: 3-23.
- 859 **Restrepo-Coupe N, da Rocha HR, Hutyrá LR, da Araujo AC, Borma LS, Christoffersen B,**
860 **Cabral OMR, de Camargo PB, Cardoso FL, da Costa ACL et al.** 2013. What drives
861 the seasonality of photosynthesis across the Amazon basin? A cross-site analysis of eddy
862 flux tower measurements from the Brasil flux network. *Agricultural and forest*
863 *meteorology*, **182**: 128-144.
- 864 **Rice AH, Pyle EH, Saleska SR, Hutyrá L, Palace M, Keller M, de Camargo PB, Portilho K,**
865 **Marques DF, Wofsy SC.** 2004. Carbon balance and vegetation dynamics in an old-
866 growth Amazonian forest. *Ecological Applications*, **14**: 55-71.
- 867 **Richardson AD, Braswell BH, Hollinger DY, Jenkins JP, Ollinger SV.** 2009. Near-surface
868 remote sensing of spatial and temporal variation in canopy phenology. *Ecological*
869 *Applications*, **19**: 1417-1428.
- 870 **Richardson AD, Anderson RS, Arain MA, Barr AG, Bohrer G, Chen G, Chen JM, Ciais P,**
871 **Davis KJ, Desai AR, et al.** 2012. Terrestrial biosphere models need better representation
872 of vegetation phenology: results from the North American Carbon Program Site
873 Synthesis. *Global Change Biology*, **18**: 566-584.
- 874 **Roberts DA, Nelson BW, Adams JB, Palmer F.** 1998. Spectral changes with leaf aging in
875 Amazon caatinga. *Trees*, **12**: 315-325.
- 876 **Saleska, S.R., J. Wu, K. Guan, A.C. Araujo, A. Huete, A.D. Nobre, N. Restrepo-Coupe.**
877 **2016.** Brief Communications Arising: Dry season greening of Amazon forests. *Nature*,
878 **531**: E4-E5.
- 879 **Schöngart J, Piedade MTF, Ludwigshausen S, Horna V, Worbes M.** 2002. Phenology and
880 stem-growth periodicity of tree species in Amazonian floodplain forests. *Journal of*
881 *Tropical Ecology*, **18**: 581-597.
- 882 **Sellers PJ, Berry JA, Collatz GJ, Field CB, Hall FG.** 1992. Canopy reflectance,
883 photosynthesis, and transpiration. III. A reanalysis using improved leaf models and a new
884 canopy integration scheme. *Remote Sensing of Environment*, **42**: 187-216

- 885 **Serbin SP, Dillaway DN, Kruger EL, Townsend PA.** 2012. Leaf optical properties reflect
886 variation in photosynthetic metabolism and its sensitivity to temperature. *Journal of*
887 *Experimental Botany*, **63**: 489-502.
- 888 **Serbin SP, Singh A, McNeil BE, Kingdon CC, Townsend PA.** 2014. Spectroscopic
889 determination of leaf morphological and biochemical traits for northern temperate and
890 boreal tree species. *Ecological Applications*, **24**: 1651-1669.
- 891 **Singh A, Serbin SP, McNeil BE, Kingdon CC, Townsend PA.** 2015. Imaging spectroscopy
892 algorithms for mapping canopy foliar chemical and morphological traits and their
893 uncertainties. *Ecological Applications*, **25**: 2180-2197.
- 894 **Terashima I, Miyazawa SI, Hanba YT.** 2001. Why are sun leaves thicker than shade leaves?—
895 Consideration based on analyses of CO₂ diffusion in the leaf. *Journal of Plant Research*,
896 **114**: 93-105.
- 897 **Toomey M, Roberts D, Nelson B.** 2009. The influence of epiphylls on remote sensing of humid
898 forests. *Remote Sensing of Environment*, **113**: 1787-1798.
- 899 **Townsend PA, Foster JR, Chastain RA, Currie WS.** 2003. Application of imaging
900 spectroscopy to mapping canopy nitrogen in the forests of the central Appalachian
901 Mountains using Hyperion and AVIRIS. *IEEE Transactions on Geoscience and Remote*
902 *Sensing*, **41**: 1347-1354.
- 903 **Tucker CJ.** 1979. Red and photographic infrared linear combinations for monitoring vegetation.
904 *Remote Sensing of Environment*, **8**: 127-150.
- 905 **Ustin SL, Gitelson AA, Jacquemoud S, Schaepman M, Asner GP, Gamon JA, Zarco-**
906 **Tejada P.** 2009. Retrieval of foliar information about plant pigment systems from high
907 resolution spectroscopy. *Remote Sensing of Environment*, **113**: S67-S77.
- 908 **Wang Y, Siemann E, Wheeler GS, Zhu L, Gu X, Ding J.** 2012. Genetic variation in anti-
909 herbivore chemical defences in an invasive plant. *Journal of Ecology*, **100**: 894-904.
- 910 **Wilson KB, Baldocchi DD, Hanson PJ.** 2001. Leaf age affects the seasonal pattern of
911 photosynthetic capacity and net ecosystem exchange of carbon in a deciduous forest.
912 *Plant, Cell & Environment*, **24**: 571-583.
- 913 **Wolter PT, Townsend PA, Sturtevant BR, Kingdon CC.** 2008. Remote sensing of the
914 distribution and abundance of host species for spruce budworm in Northern Minnesota
915 and Ontario. *Remote Sensing of Environment*, **112**: 3971-3982.
- 916 **Wold S, Sjöström M, Eriksson L.** 2001. PLS-regression: a basic tool of
917 chemometrics. *Chemometrics and intelligent laboratory systems*, **58**: 109-130.
- 918 **Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J,**
919 **Chapin T, Cornelissen JHC, Diemer M, et al.** (2004). The worldwide leaf economics
920 spectrum. *Nature*, **428**: 821-827.
- 921 **Xu QS, Liang YZ.** 2001. Monte Carlo cross validation. *Chemometrics and Intelligent*
922 *Laboratory Systems*, **56**: 1-11.
- 923 **Yang X, Tang J, Mustard JF.** 2014. Beyond leaf color: Comparing camera-based phenological
924 metrics with leaf biochemical, biophysical, and spectral properties throughout the
925 growing season of a temperate deciduous forest. *Journal of Geophysical Research:*
926 *Biogeosciences*, **119**: 181-191.
- 927 **Wu J, Albert LP, Lopes AP, Restrepo-Coupe N, Hayek M, Wiedemann K, Guan K, Stark**
928 **SC, Christoffersen B, Prohaska N, et al.** 2016. Leaf development and demography
929 explain photosynthetic seasonality in Amazonian evergreen forests. *Science*, **351**: 972-
930 976.

931 **Table 1.** Tree-environment combinations and associated canopy environments for leaf traits and
 932 spectra measurements at two tropical forests in Brazil and Peru. (Tree-environment combinations
 933 were coded by “*species name_canopy position*”, with four canopy position codes: ‘SU’ sunlit,
 934 ‘SH’ shaded, ‘MC’ mid-canopy, and ‘US’ understory).

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	<i>E. uncinatum</i> _SU	<i>Erisma uncinatum</i> Warm.	Vochysiaceae	1.48	Canopy Sun	39.0	1.0	192.60	49.63
Brazil	<i>E. uncinatum</i> _SH	<i>Erisma uncinatum</i> Warm.	Vochysiaceae	1.48	Canopy Shade	30.0	10.0	130.79	48.33
Brazil	<i>Ocotea</i> _SU	<i>Ocotea</i> _sp.	Lauraceae	0.73	Canopy Sun	37.0	1.0	131.37	47.57
Brazil	<i>Ocotea</i> _SH	<i>Ocotea</i> _sp.	Lauraceae	0.73	Canopy Shade	32.5	5.5	91.17	45.28
Brazil	<i>M.huberi</i> _SU	<i>Manilkara huberi</i> (Ducke) A. Chev.	Sapotaceae	0.92	Canopy Sun	37.5	0.5	229.94	49.18
Brazil	<i>M.huberi</i> _SH	<i>Manilkara huberi</i> (Ducke) A. Chev.	Sapotaceae	0.92	Canopy Shade	31.2	6.8	218.25	50.39
Brazil	<i>C.scleroxylon</i> _SU	<i>Chamaecrista scleroxylon</i> (Ducke) H.S.Irwin & Barneby	Leguminosae-Caesalpinioideae	0.47	Canopy Sun	24.5	1.0	80.47	49.79
Brazil	<i>C.scleroxylon</i> _SH	<i>Chamaecrista scleroxylon</i> (Ducke) H.S.Irwin & Barneby	Leguminosae-Caesalpinioideae	0.47	Canopy Shade	20.0	5.5	59.31	55.74
Brazil	<i>E.uchi</i> _MC	<i>Endopleura uchi</i> (Huber) Cuatrec.	Humiriaceae	0.46	Middle Canopy	24.5	16.5	114.19	46.01
Brazil	<i>E.coriacea</i> _MC	<i>Eschweilera coriacea</i>	Lecythidaceae	0.25	Middle Canopy	22.8	15.2	122.01	44.63
Brazil	<i>H.courbaril</i> _MC	<i>Hymenaea courbaril</i> L.	Leguminosae-Caesalpinioideae	0.45	Middle Canopy	30.0	11.0	117.21	52.02
Brazil	<i>Miconia</i> _SU	<i>Miconia</i> -sp.	Melastomataceae	0.14	Understory	13.7	29.3	58.62	61.37
Brazil	<i>G.amazonicum</i> _US	<i>Glycydendron amazonicum</i> (Ducke)	Euphorbiaceae	0.11	Understory	7.7	33.3	54.74	62.96
Brazil	<i>M.ruficalyx</i> _US	<i>Miconia ruficalyx</i> Gleason	Melastomataceae	0.14	Understory	13.5	12.0	54.73	59.8
Brazil	<i>V.elongate</i> _US	<i>Virola elongata</i> (Benth.) Warb.	Myristicaceae	0.17	Understory	19.0	6.5	75.65	59.84
Peru	<i>L.brittoniana</i> _SU	<i>Licania brittoniana</i>	Chrysobalanaceae	1.88	Canopy Sun	29.5	0.5	121.09	49.89
Peru	<i>Q.simaruba</i> _SU	<i>Quassia simaruba</i>	Simaroubaceae	1.62	Canopy Sun	29.0	0.5	188.04	44.49
Peru	<i>R.ovale</i> _SU	<i>Ruizodendron ovale</i>	Annonaceae	1.23	Canopy Sun	32.6	0.5	91.27	50.53
Peru	<i>A.parvifolium</i> _SU	<i>Aspidosperma parvifolium</i>	Apocynaceae	1.57	Canopy Sun	33.7	0.5	110.94	55.09
Peru	<i>C.macrosperma</i> _SU	<i>Couratari macrosperma</i>	Lecythidaceae	1.43	Canopy Sun	33.7	0.5	170.65	45.78
Peru	<i>L.longistyla</i> _SU	<i>Licania longistyla</i>	Chrysobalanaceae	1.43	Canopy Sun	30.3	0.5	125.87	37.70
Peru	<i>B.excelsa</i> _SU	<i>Bertholletia excelsa</i>	Lecythidaceae	2.30	Canopy Sun	34.0	0.5	147.93	45.07
Peru	<i>T.chrysaloides</i> _SU	<i>Tachigali chrysaloides</i>	Caesalpinioideae	2.10	Canopy Sun	29.7	0.5	180.51	47.29
Peru	<i>C.racemosa</i> _SU	<i>Clarisia racemosa</i>	Moraceae	1.58	Canopy Sun	33.8	0.5	120.86	49.76
Peru	<i>E.coriacea</i> _SU	<i>Eschweilera coriacea</i>	Lecythidaceae	1.88	Canopy Sun	33.3	0.5	157.55	46.61
Peru	<i>G.boliviana</i> _SU	<i>Gautteria boliviana</i>	Annonaceae	1.17	Canopy Sun	31.9	0.5	113.31	52.22
Peru	<i>P.franciscana</i> _SU	<i>Pouteria franciscana</i>	Sapotaceae	2.37	Canopy Sun	35.4	0.5	124.72	48.31

935 DBH, diameter at breast height; LMA, leaf mass per area; LWC, leaf water content; Old: leaf
 936 age greater than 300 days (and senescent leaves were excluded).

937
 938

939 **Figure Legends**

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

945
 946 **Figure 2.** Leaf trait variation with leaf age and canopy environment at the Brazil site for 11 tree-
 947 environment combinations (see Table 1): **(a)** Leaf Mass per Area (LMA), and **(b)** Leaf Water
 948 Content (LWC). Each colored line represents a tree in a particular environment (indicated by a
 949 “*species name_canopy position*” label in the legend, where “canopy position” is represented by
 950 four codes: ‘SU’ sunlit, ‘SH’ shaded, ‘MC’ mid-canopy, and ‘US’ understory). Solid colored
 951 lines indicate canopy sunlit environment ($R^2=0.31$ for LMA and $R^2=0.60$ for LWC) and canopy
 952 shade environment ($R^2=0.23$ for LMA and $R^2=0.80$ for LWC), dashed lines indicate mid-canopy
 953 (MC, $R^2=0.76$ for LMA and $R^2=0.74$ for LWC) or understory (US, $R^2=0.39$ for LMA and
 954 $R^2=0.61$ for LWC), and black lines indicate community average relationships: R^2 is the
 955 proportion of variation in the trait that is explained by leaf age (model: $\log(\text{trait}) = a * \log(\text{age})$
 956 $+b$). *** indicates $p < 10^{-5}$.

957
 958 **Figure 3.** Leaf age and canopy environment induced spectra variation at the Brazil site. **(a)** Age-
 959 dependent leaf level hyperspectral reflectance across all canopy environments for: young (blue
 960 lines), mature (green lines), and old (red lines) leaves (mean value in solid lines with shaded 95%
 961 confidence interval); **(b)** Canopy environment-dependent leaf level hyperspectral reflectance
 962 across all leaf ages for: canopy sun (in red lines; $n=4$ trees), canopy shade (in purple lines; $n=4$),
 963 mid-canopy (in green lines; $n=3$), and understory (in blue lines; $n=4$); **(c)** Normalized differences
 964 of young, mature, and old leaf spectra from the mean leaf spectra (solid lines +/- shaded 95%
 965 confidence interval); **(d)** Normalized environment differences of canopy sun, canopy shade, mid-
 966 canopy and understory leaf spectra from the mean leaf spectra (solid lines +/- shaded 95%
 967 confidence interval). Note: Normalized difference = (mean reflectance within age scenario –
 968 mean reflectance across all scenarios)/standard deviation of reflectance across all scenarios.

969

970 **Figure 4.** Cross-site spectra-age model results for data from both Peruvian and Brazilian sites,
 971 based on fitting variation of the Peru reference Partial Least Squares Regression (PLSR) models
 972 to a subset of observations at the Peru site only (i.e., the Peru training dataset): **(a):** Root Mean
 973 Square Error (RMSE) between observed and modeled leaf age plotted against the number of
 974 latent variables incorporated for PLSR models; **(b):** The proportion of variation in leaf age
 975 explained by PLSR models (R^2) plotted against the number of latent variables incorporated.
 976 Different symbols in (a) and (b) represent different datasets, as indicated (see also Table 1): the
 977 performance of the original 7-latent variable model for each dataset is indicated by the points in
 978 the blue shaded box in **a, b**); an optimal model for prediction across sites (RMSE minimized and
 979 R^2 maximized for Brazil validation datasets not used in model fitting) emerges for 5 latent
 980 variables (gray shaded box in **a, b**). **(c):** Spectral regression coefficients for the optimized PLSR
 981 model with 5 latent variables; **(d):** Variable Importance in Projection (VIP) for the optimized
 982 PLSR model with 5 latent variables (spectral features greater than 0.8 represent the important
 983 spectral regions for leaf age modeling).

984
 985 **Figure 5.** Example of developmental trajectory in a mid-canopy tree in Brazil (*E.uchi_MC*),
 986 including: **(a)** the appearance of leaves versus age in RGB photos, showing reddish leaves when
 987 leaf ages are 40 days or younger; **(b)** the aging of leaves as revealed by leaf reflectance
 988 hyperspectra (measured by an ASD spectroradiometer); and **(c)** comparison between spectra-age
 989 model coefficients of seven latent variables (in red) and five latent variables (in black), (see Fig.
 990 4; as derived from the model parameterized by Peruvian sunlit canopy leaves, which do not have
 991 reddish young leaves). Coefficients in the spectral region marked by the blue window are near-
 992 zero in the 5-variable model, which more accurately predicts leaf age in three mid-canopy or
 993 understory trees: (d) *E.uchi_MC*, (e) *G. amazonicum_US*, and (f) *M. ruficalyx_US*). The non-
 994 zero coefficients in the 7-variable model make it more sensitive to reddish shifts in the
 995 understory Brazilian leaves, a confounding effect which causes significant over-prediction of
 996 young leaf age in these same trees when the 7-variable model is applied (in g, h, i).

997
 998 **Figure 6. (a)** Branch height probability distribution for Peru (grey line) and Brazil (dark green),
 999 with color symbols indicating branch height for each sample; **(b)** Leaf trait scatter plot showing
 1000 leaf water content (LWC) versus leaf mass per area (LMA) for Peru and Brazil leaf samples from

1001 the old leaf age class (Table 1). Red rectangles in (a) and (b) bound the sample space for mid- to
 1002 upper canopy leaves from Peru and Brazil sites.

1003

1004 **Figure 7.** Performance (observed versus predicted leaf age) of the optimal Peru-trained spectral
 1005 leaf age model (Fig 4, 5-variable model) as applied to Brazilian samples from four canopy
 1006 environments **(a)-(d)**: canopy sun; **(e)-(h)**: canopy shade; **(i)-(k)**: mid-canopy; **(l)-(o)**: understory.
 1007 OLS regressions (black lines) quantify the deviation of the scatterplots from the ideal 1:1 line
 1008 (dashed lines). R^2 quantifies the fit of the regression line – i.e., the variation in leaf age explained
 1009 by combining the Peru leaf age model (applied to the whole dataset) with the individual tree
 1010 regressions – and RMSE is the corresponding Root Mean Square Error.

1011

1012 **Figure 8.** The regression line slopes and intercepts of each tree-environment combination (from
 1013 Fig. 7) plotted against branch height and leaf mass per area (LMA) for the Brazil site: **(a)** Slope
 1014 vs. branch height; **(b)** Slope vs. LMA; **(c)** Intercept vs. branch height; **(d)** Intercept vs. LMA.

1015

1016 **Figure 9.** Performance of leaf age models for the Brazilian leaf samples under four scenarios: **(a)**
 1017 the Peru model (parameterized by using Peruvian leaf spectra only; the same model as presented
 1018 in Chavana-Bryant *et al.*, 2016 using seven latent variables); **(b)** the Peru reference model
 1019 (parameterized by using Peruvian leaf spectra only); **(c)** the “Peru Spectra+all Trait” model
 1020 (parameterized by using Peruvian leaf spectra and traits); **(d)** the “All Spectra” model
 1021 (parameterized by using both Brazilian and Peruvian leaf spectra); **(e)** the “All Spectra+ all Trait”
 1022 model (or parameterized by using both Brazilian and Peruvian leaf spectra and traits). Four
 1023 different color circles represent the leaf samples from Brazil canopy sun (red circles; n=4 trees),
 1024 Brazil canopy shade (yellow circles; n=4), Brazil mid-canopy (green circles; n=3), and Brazil
 1025 understory trees (blue circles; n=4). Four different color lines represent the corresponding
 1026 ordinary least regression (OLS) between predicted and observed leaf ages; central grey line
 1027 represents the OLS analysis for all Brazil samples. The “All Spectra” model (d) is our
 1028 “recommended” general model. The number of optimal latent variables in panel b was identified
 1029 in Fig. 4, and in panels c to e were identified in Fig. S6

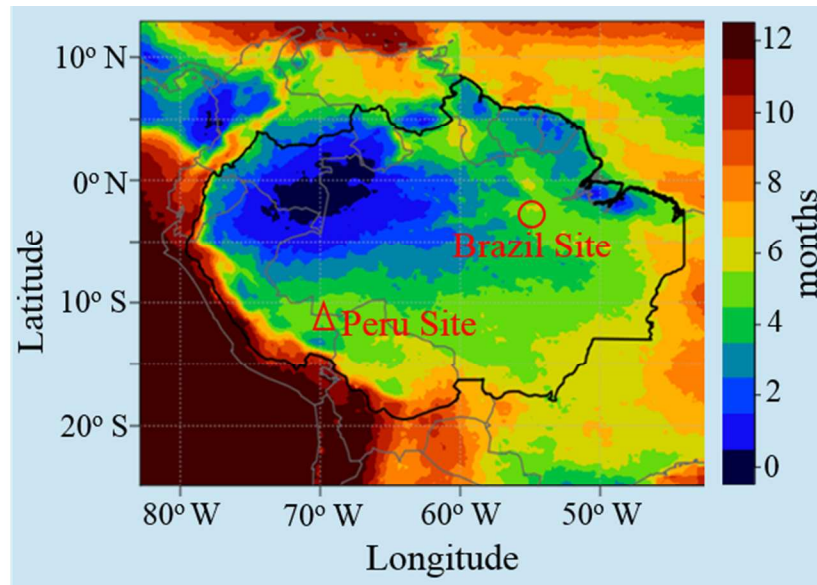
1030

1031

1032

Main Figures and Tables

1033 **Figure 1.** Location of sites in the Amazon basin, including the Tapajós National Forest in Brazil
1034 (red circle) and the Tambopata National Reserve in Peru (red triangle). The black curve indicates
1035 the boundary of the Amazon basin. The background is a map of dry season length (in months;
1036 see color legend), which is derived from Tropical Rainfall Measuring Mission (TRMM) satellite
1037 data from 1998 to 2013.

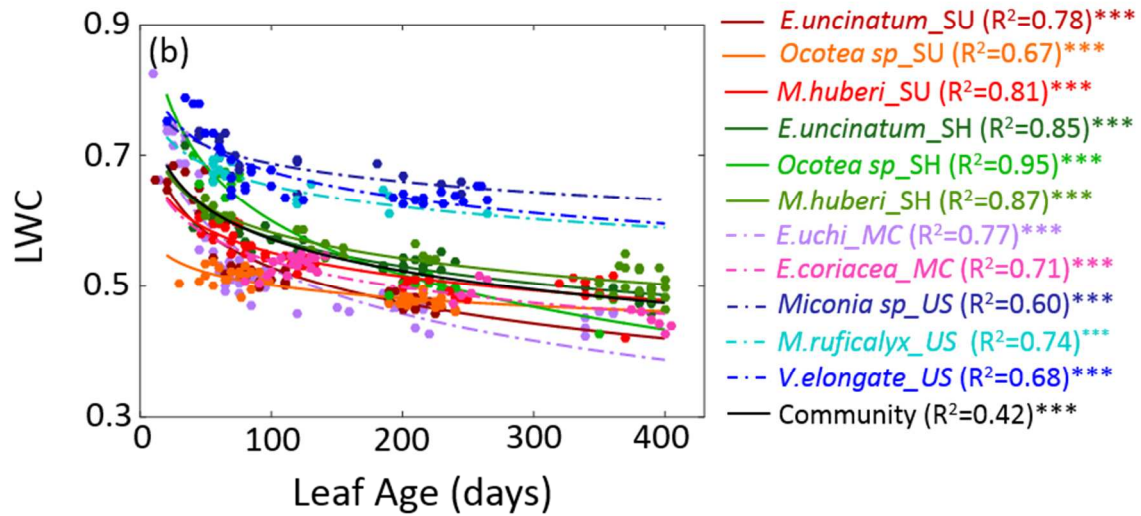
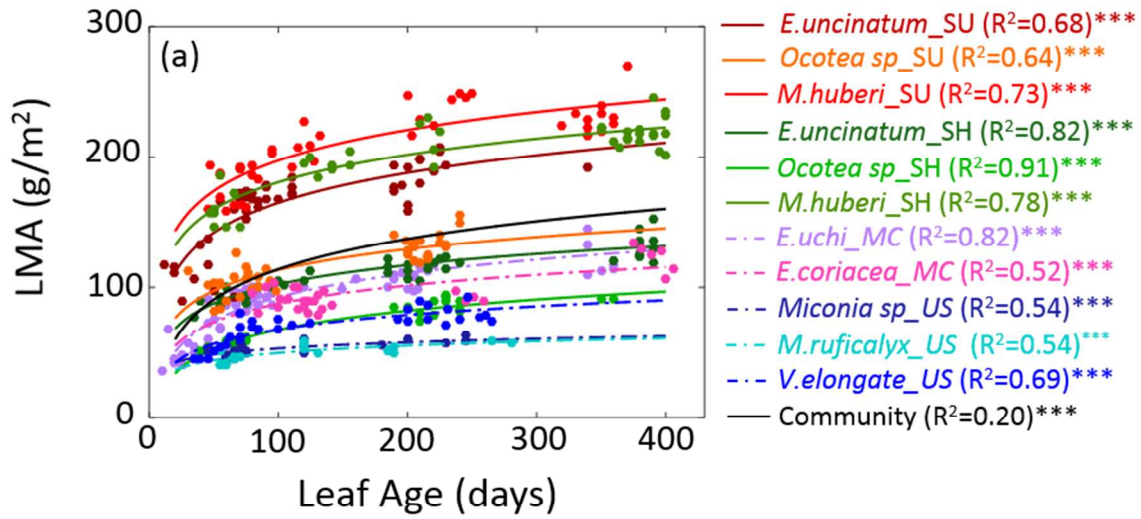


1038

1039

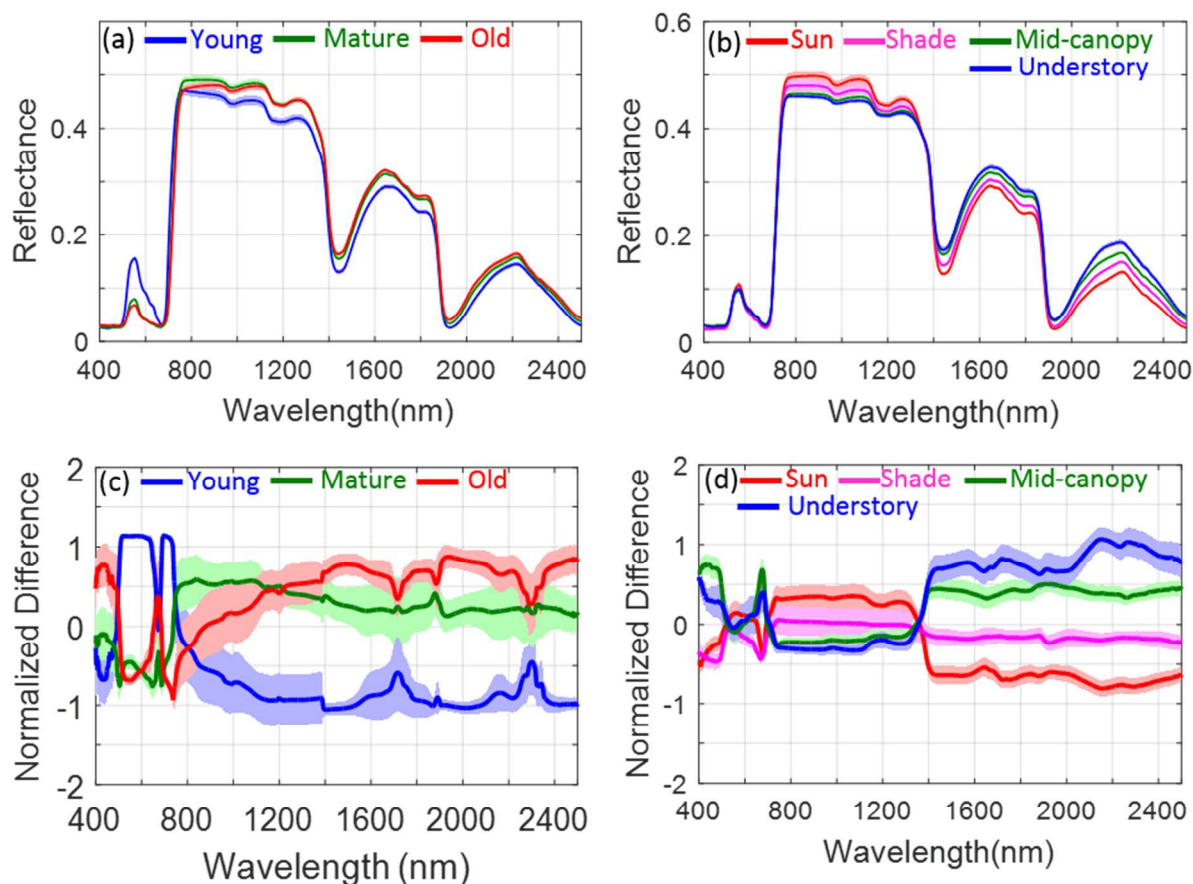
1040

1041 **Figure 2.** Leaf trait variation with leaf age and canopy environment at the Brazil site for 11 tree-
1042 environment combinations (see Table 1): **(a)** Leaf Mass per Area (LMA), and **(b)** Leaf Water
1043 Content (LWC). Each colored line represents a tree in a particular environment (indicated by a
1044 “*species name_canopy position*” label in the legend, where “canopy position” is represented by
1045 four codes: ‘SU’ sunlit, ‘SH’ shaded, ‘MC’ mid-canopy, and ‘US’ understory). Solid colored
1046 lines indicate canopy sunlit environment ($R^2=0.31$ for LMA and $R^2=0.60$ for LWC) and canopy
1047 shade environment ($R^2=0.23$ for LMA and $R^2=0.80$ for LWC), dashed lines indicate mid-canopy
1048 (MC, $R^2=0.76$ for LMA and $R^2=0.74$ for LWC) or understory (US, $R^2=0.39$ for LMA and
1049 $R^2=0.61$ for LWC), and black lines indicate community average relationships: R^2 is the
1050 proportion of variation in the trait that is explained by leaf age (model: $\log(\text{trait}) = a * \log(\text{age})$
1051 +b). *** indicates $p < 10^{-5}$.



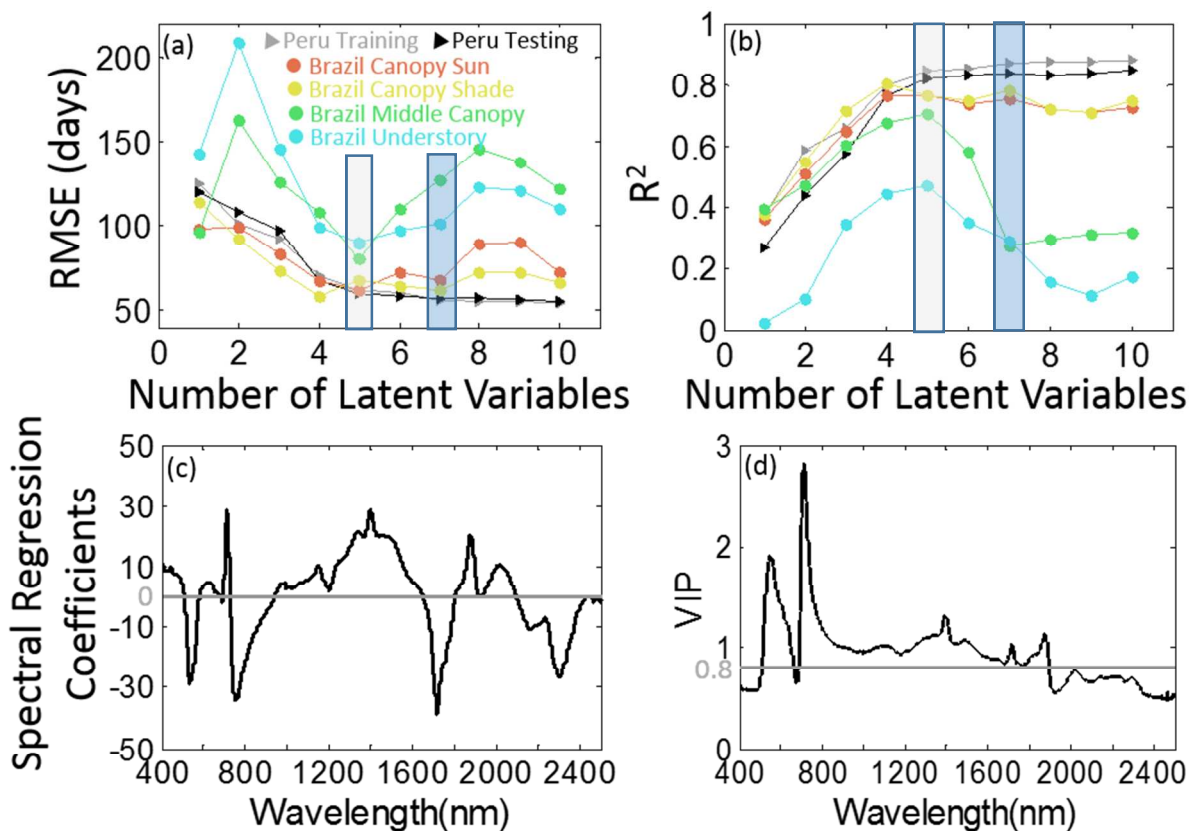
1052

1053 **Figure 3.** Leaf age and canopy environment induced spectra variation at the Brazil site. **(a)** Age-
 1054 dependent leaf level hyperspectral reflectance across all canopy environments for: young (blue
 1055 lines), mature (green lines), and old (red lines) leaves (mean value in solid lines with shaded 95%
 1056 confidence interval); **(b)** Canopy environment-dependent leaf level hyperspectral reflectance
 1057 across all leaf ages for: canopy sun (in red lines; n=4 trees), canopy shade (in purple lines; n=4),
 1058 mid-canopy (in green lines; n=3), and understory (in blue lines; n=4); **(c)** Normalized differences
 1059 of young, mature, and old leaf spectra from the mean leaf spectra (solid lines +/- shaded 95%
 1060 confidence interval); **(d)** Normalized environment differences of canopy sun, canopy shade, mid-
 1061 canopy and understory leaf spectra from the mean leaf spectra (solid lines +/- shaded 95%
 1062 confidence interval). Note: Normalized difference = (mean reflectance within each scenario –
 1063 mean reflectance across all scenarios)/standard deviation of reflectance across all scenarios.



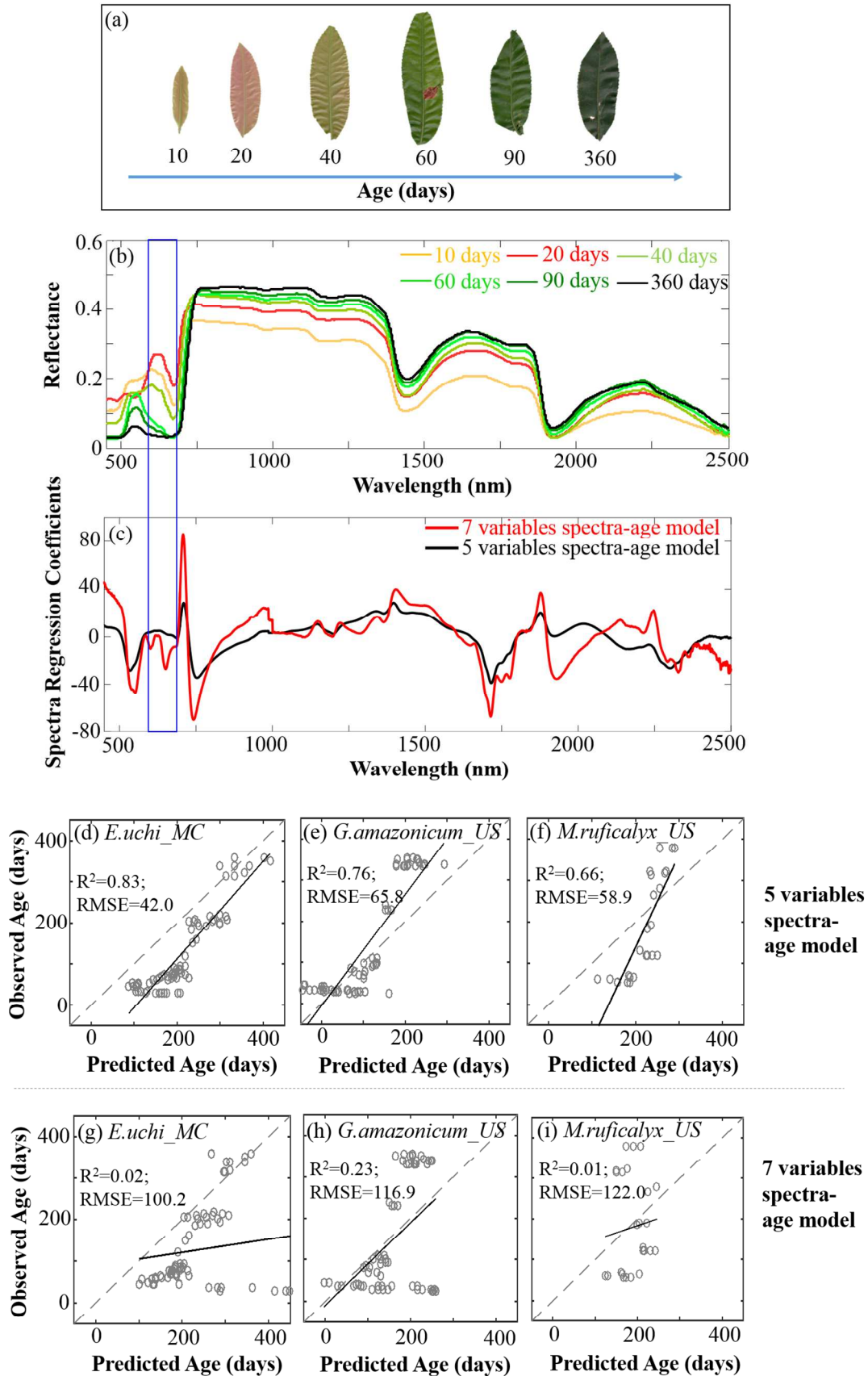
1064
 1065
 1066

1067 **Figure 4.** Cross-site spectra-age model results for data from both Peruvian and Brazilian sites,
 1068 based on fitting variation of the Peru reference Partial Least Squares Regression (PLSR) models
 1069 to a subset of observations at the Peru site only (i.e., the Peru training dataset): **(a):** Root Mean
 1070 Square Error (RMSE) between observed and modeled leaf age plotted against the number of
 1071 latent variables incorporated for PLSR models; **(b):** The proportion of variation in leaf age
 1072 explained by PLSR models (R^2) plotted against the number of latent variables incorporated.
 1073 Different symbols in (a) and (b) represent different datasets, as indicated (see also Table 1): the
 1074 performance of the original 7-latent variable model for each dataset is indicated by the points in
 1075 the blue shaded box in **a, b**); an optimal model for prediction across sites (RMSE minimized and
 1076 R^2 maximized for Brazil validation datasets not used in model fitting) emerges for 5 latent
 1077 variables (gray shaded box in **a, b**). **(c):** Spectral regression coefficients for the optimized PLSR
 1078 model with 5 latent variables; **(d):** Variable Importance in Projection (VIP) for the optimized
 1079 PLSR model with 5 latent variables (spectral features greater than 0.8 represent the important
 1080 spectral regions for leaf age modeling).

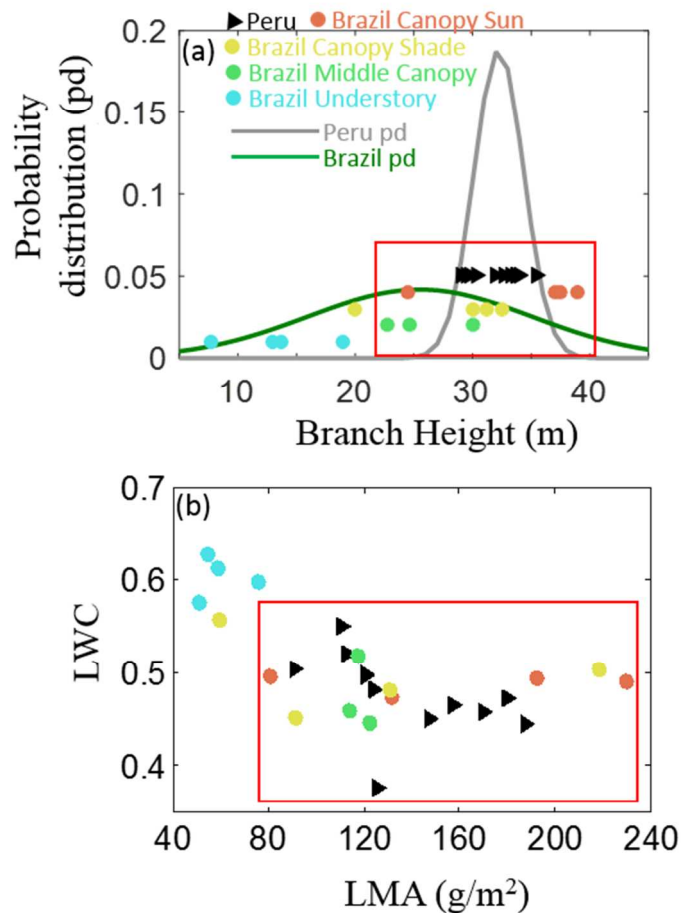


1081
 1082
 1083

1084 **Figure 5.** Example of developmental trajectory in a mid-canopy tree in Brazil (*E.uchi_MC*),
1085 including: **(a)** the appearance of leaves versus age in RGB photos, showing reddish leaves when
1086 leaf ages are 40 days or younger; **(b)** the aging of leaves as revealed by leaf reflectance
1087 hyperspectra (measured by an ASD spectroradiometer); and **(c)** comparison between spectra-age
1088 model coefficients of seven latent variables (in red) and five latent variables (in black), (see Fig.
1089 4; as derived from the model parameterized by Peruvian sunlit canopy leaves, which do not have
1090 reddish young leaves). Coefficients in the spectral region marked by the blue window are near-
1091 zero in the 5-variable model, which more accurately predicts leaf age in three mid-canopy or
1092 understory trees: (d) *E.uchi_MC*, (e) *G. amazonicum_US*, and (f) *M. ruficalyx_US*). The non-
1093 zero coefficients in the 7-variable model make it more sensitive to reddish shifts in the
1094 understory Brazilian leaves, a confounding effect which causes significant over-prediction of
1095 young leaf age in these same trees when the 7-variable model is applied (in g, h, i).
1096

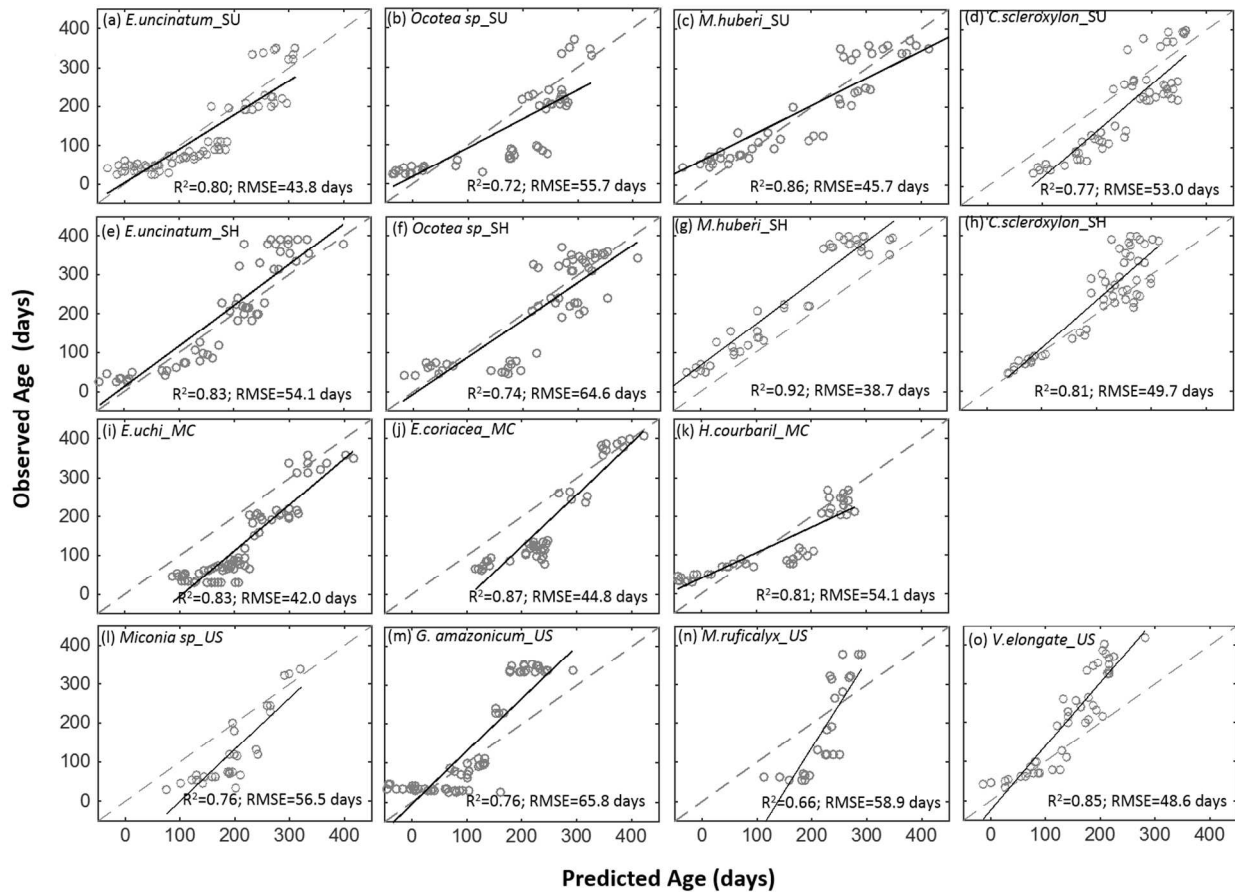


1098 **Figure 6. (a)** Branch height probability distribution for Peru (grey line) and Brazil (dark green),
 1099 with color symbols indicating branch height for each sample; **(b)** Leaf trait scatter plot showing
 1100 leaf water content (LWC) versus leaf mass per area (LMA) for Peru and Brazil leaf samples from
 1101 the old leaf age class (Table 1). Red rectangles in (a) and (b) bound the sample space for mid- to
 1102 upper canopy leaves from Peru and Brazil sites.



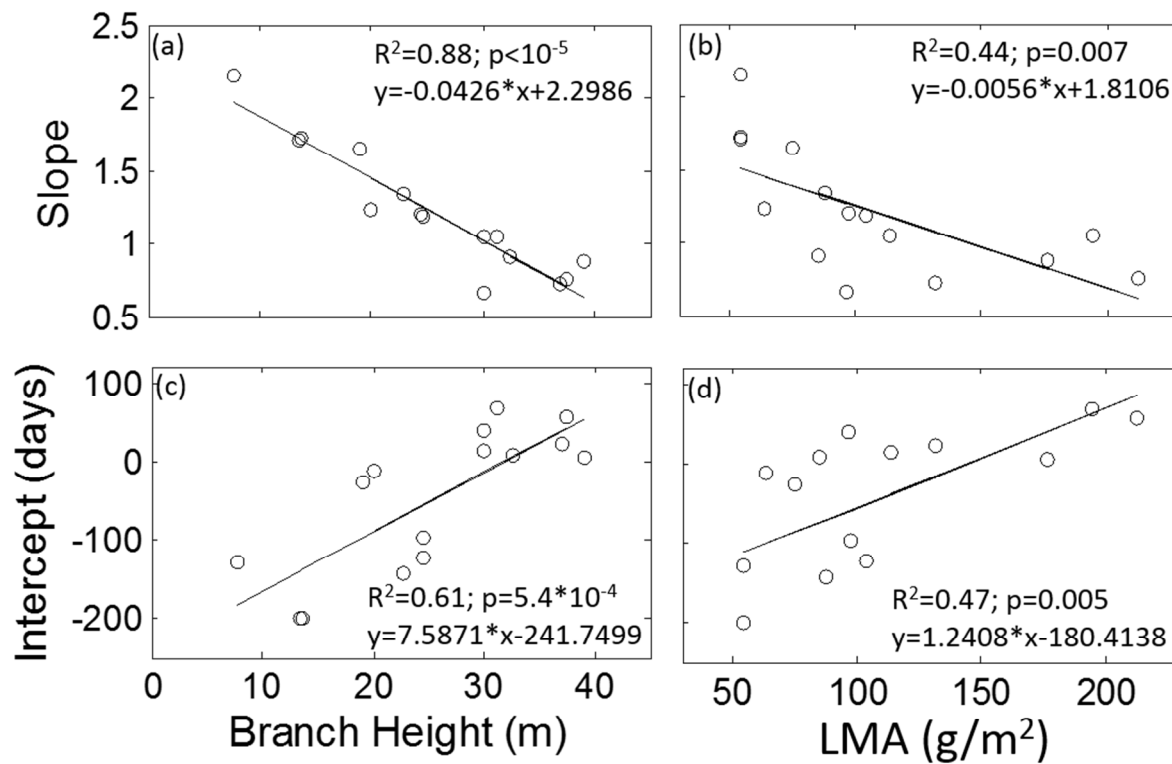
1103
 1104
 1105

1106 **Figure 7.** Performance (observed versus predicted leaf age) of the optimal Peru-trained spectral
 1107 leaf age model (Fig 4, 5-variable model) as applied to Brazilian samples from four canopy
 1108 environments **(a)-(d):** canopy sun; **(e)-(h):** canopy shade; **(i)-(k):** mid-canopy; **(l)-(o):** understory.
 1109 OLS regressions (black lines) quantify the deviation of the scatterplots from the ideal 1:1 line
 1110 (dashed lines). R^2 quantifies the fit of the regression line – i.e., the variation in leaf age explained
 1111 by combining the Peru leaf age model (applied to the whole dataset) with the individual tree
 1112 regressions – and RMSE is the corresponding Root Mean Square Error.



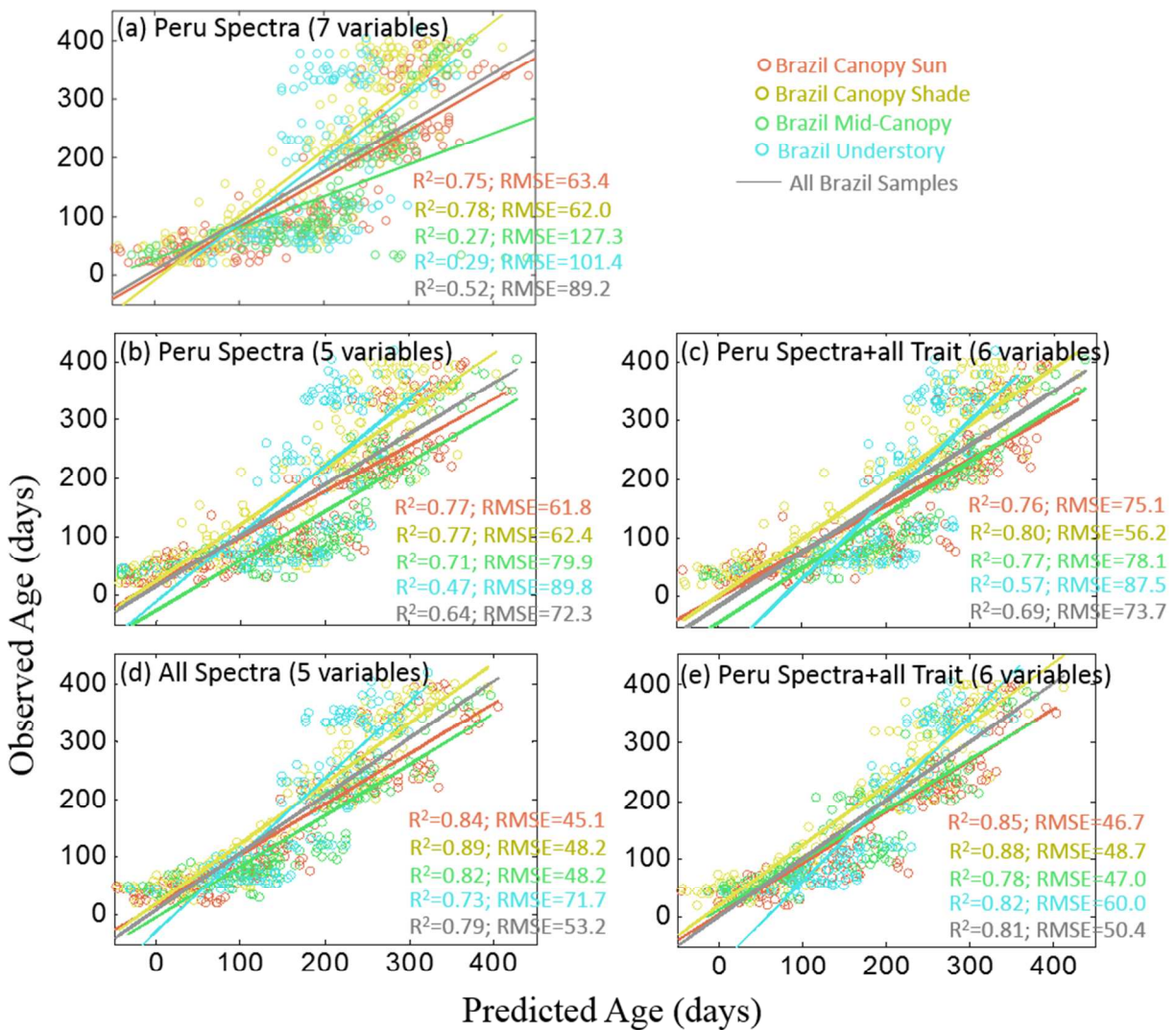
1113

1114 **Figure 8.** The regression line slopes and intercepts of each tree-environment combination (from
 1115 Fig. 7) plotted against branch height and leaf mass per area (LMA) for the Brazil site: **(a)** Slope
 1116 vs. branch height; **(b)** Slope vs. LMA; **(c)** Intercept vs. branch height; **(d)** Intercept vs. LMA.
 1117



1118
 1119

1120 **Figure 9.** Performance of leaf age models for the Brazilian leaf samples under four scenarios: **(a)**
1121 the Peru model (parameterized by using Peruvian leaf spectra only; the same model as presented
1122 in Chavana-Bryant *et al.*, 2016 using seven latent variables); **(b)** the Peru reference model
1123 optimized for multiple environments (parameterized by using Peruvian leaf spectra only); **(c)** the
1124 “Peru Spectra+all Trait” model (parameterized by using Peruvian leaf spectra and traits); **(d)** the
1125 “All Spectra” model (parameterized by using both Brazilian and Peruvian leaf spectra); **(e)** the
1126 “All Spectra+ all Trait” model (parameterized by using both Brazilian and Peruvian leaf spectra
1127 and traits). Four different color circles represent the leaf samples from Brazil canopy sun (red
1128 circles; n=4 trees), Brazil canopy shade (yellow circles; n=4), Brazil mid-canopy (green circles;
1129 n=3), and Brazil understory trees (blue circles; n=4). Four different color lines represent the
1130 corresponding ordinary least regression (OLS) between predicted and observed leaf ages; central
1131 grey line represents the OLS analysis for all Brazil samples. The “All Spectra” model (d) is our
1132 “recommended” general model. The number of optimal latent variables in panel b was identified
1133 in Fig. 4, and in panels c to e were identified in Fig. S6



1134

1135

1136 ***New Phytologist* Supporting Information**

1137 **Article title:** Convergence in relations among leaf traits, spectra and age across diverse canopy
1138 environments and two contrasting tropical forests

1139

1140 **Authors:** Jin Wu, Cecilia Chavana-Bryant, Neill Prohaska, Shawn P. Serbin, Kaiyu Guan, Loren
1141 P. Albert, Xi Yang, Willem J.D. van Leeuwen, Anthony John Garnello, Giordane Martins,
1142 Yadvinder Malhi, France Gerard, Raimundo Cosme Oliviera, and Scott R. Saleska

1143

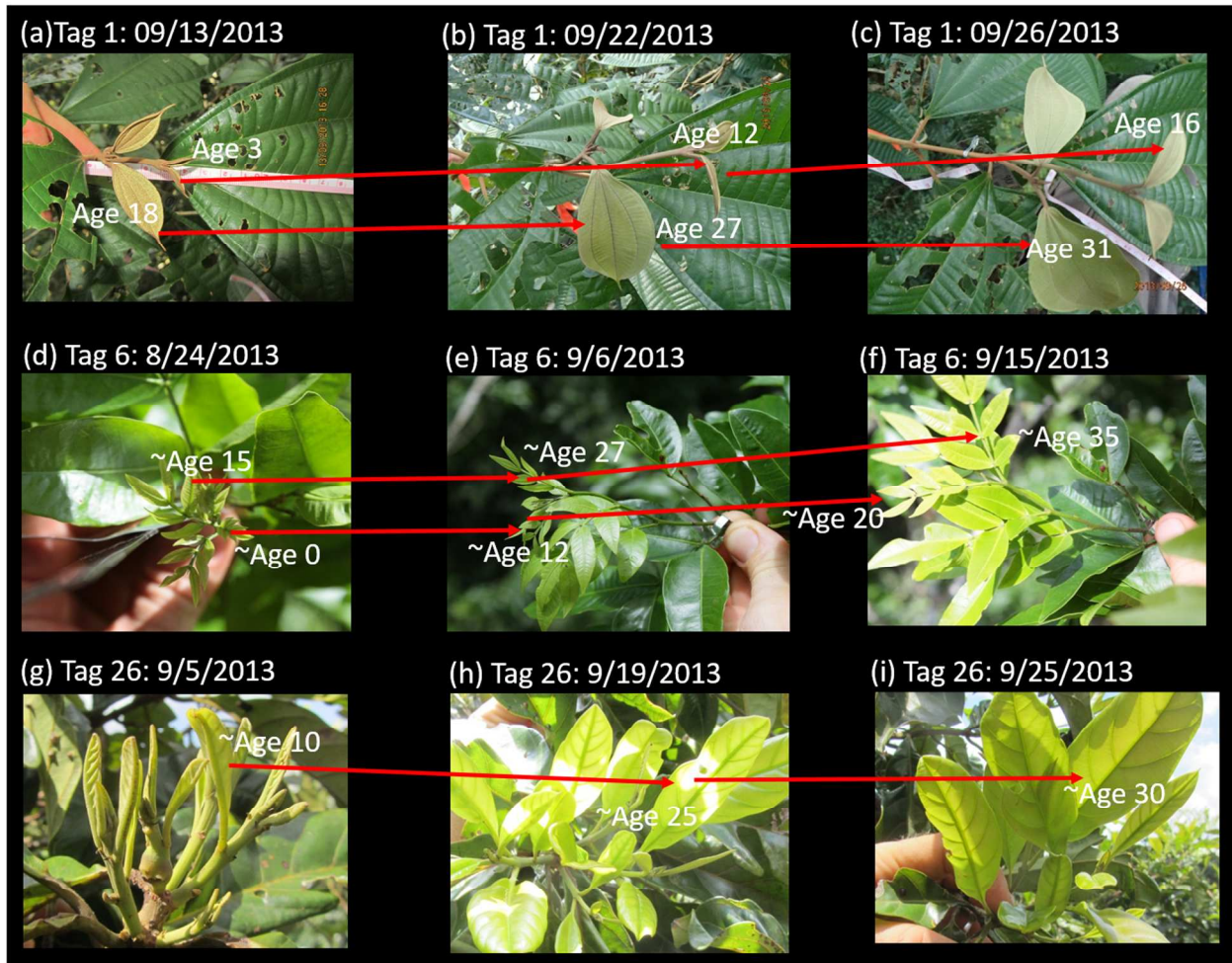
1144

1145

1146

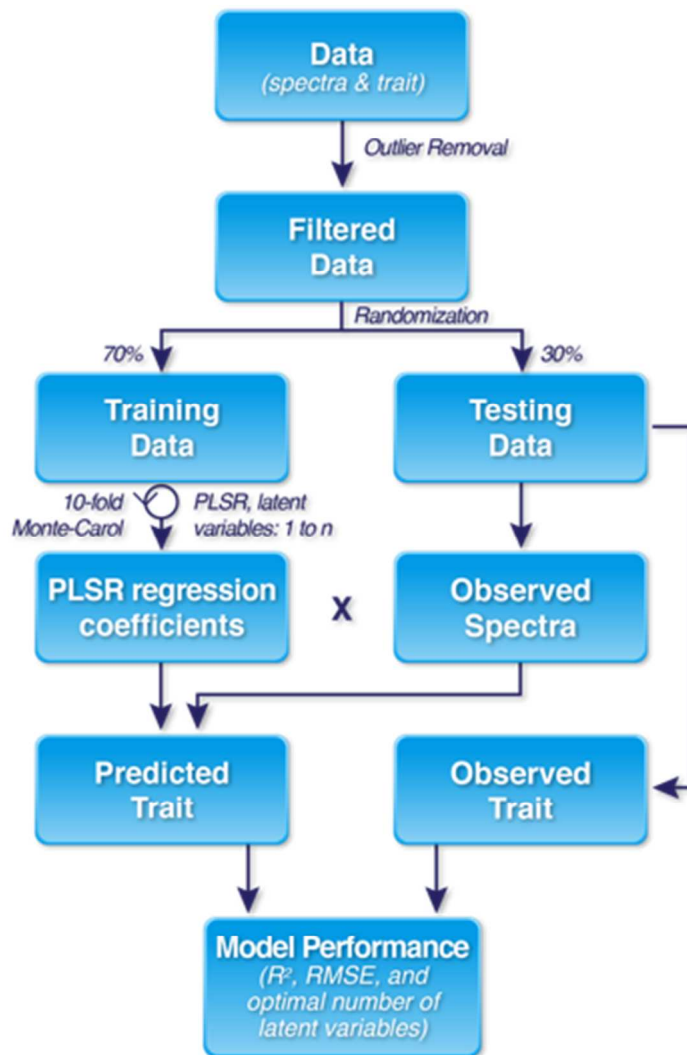
1147 **The following supporting information is available for this article:**

1148 **Figure S1.** Leaf age monitoring at the Brazil site was carried out by using metal tags and *in-situ*
 1149 photo-documentation. *In-situ* photographs acquired on three different dates illustrate leaf
 1150 development with age (dates shown are critical time periods for significant changes in leaf size
 1151 and color as leaf aged) for *M.ruficalyx* (a-c; Table 1), for *C.scleroxylon* (d-f; Table 1), and for
 1152 *E.uncinatum*. (g-i; Table 1).



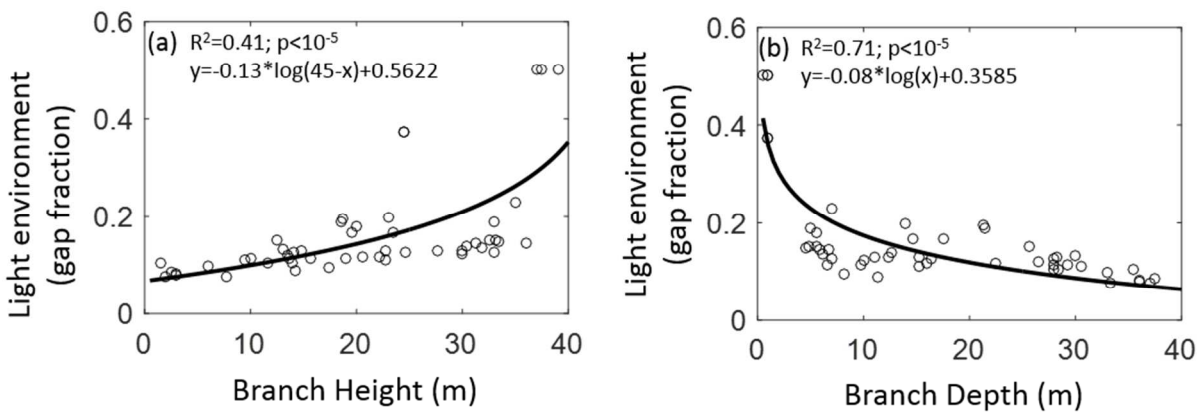
1153
 1154
 1155
 1156
 1157
 1158
 1159
 1160
 1161

1162 **Figure S2.** Flow-chart for spectra-trait analysis by using Partial Least Squares Regression
1163 (PLSR).



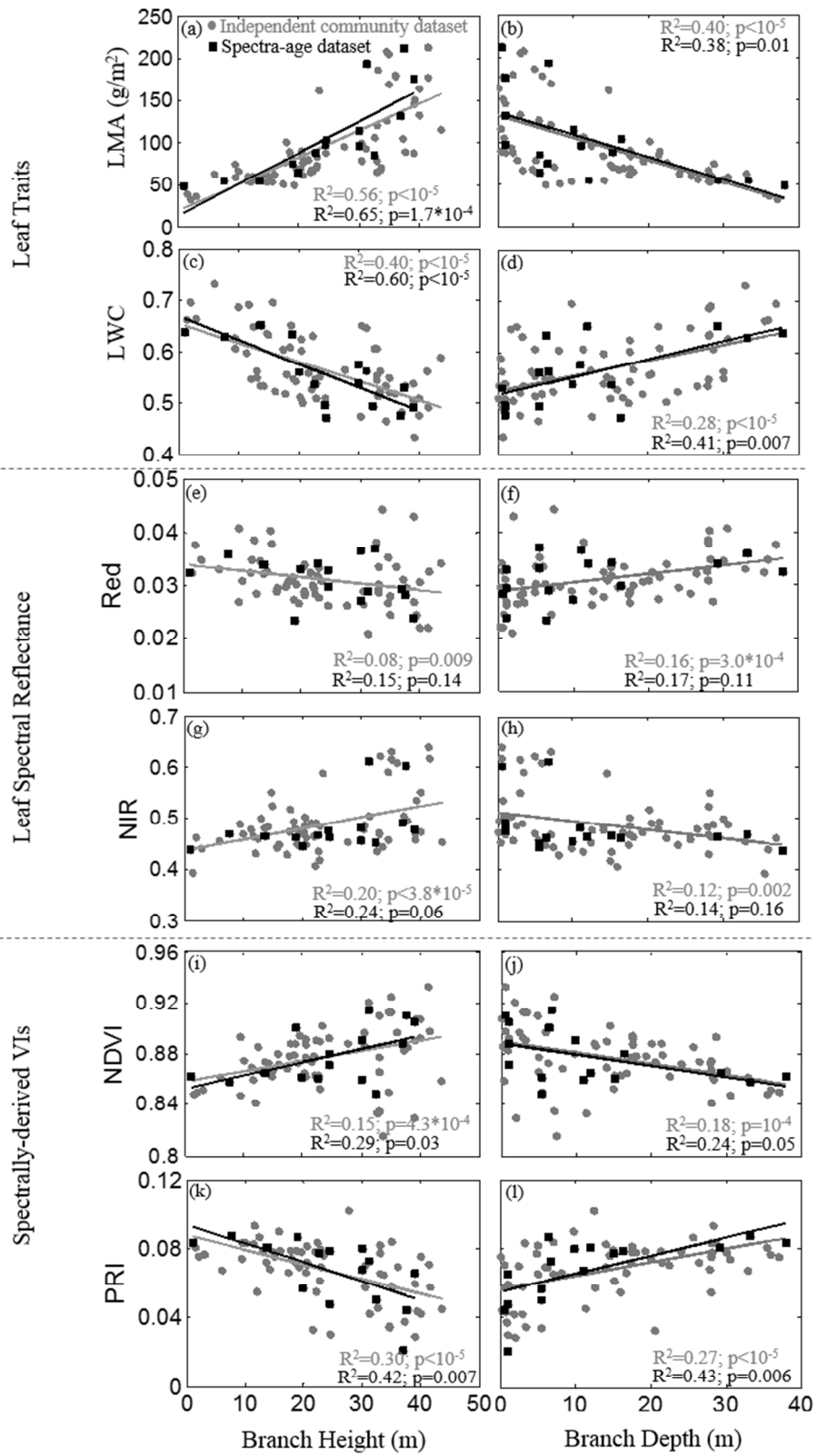
1164
1165
1166
1167

1168 **Figure S3.** *In situ* Leaf light environments (approximated by gap fraction, derived from
 1169 hemispherical photos taken above each sampled branch) versus (a) branch height above the
 1170 ground, and (b) branch depth below the local canopy top. Each point represents one tree-
 1171 environment combination. Branch depth is a significantly better proxy of light environment
 1172 ($R^2=0.71$) than branch height above the ground is ($R^2=0.41$).

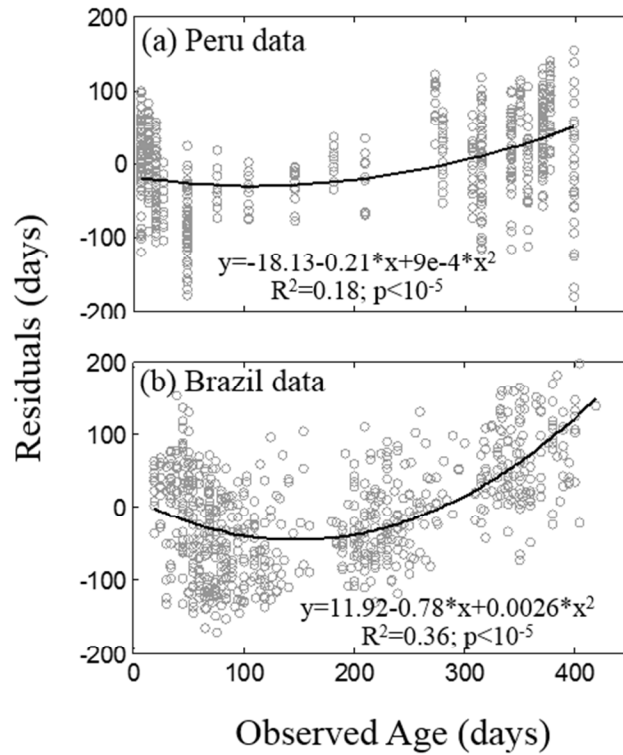


1173
 1174

1175 **Figure S4.** Mature leaf traits versus branch height of sampled leaves above the ground (left
1176 column) and branch depth (a strong proxy of light environment, see Fig. S3) of sampled leaves
1177 below the local canopy top (right column) at the Brazil site. Traits are: **(a-b)** leaf mass per area
1178 (LMA), **(c-d)** leaf water content (LWC), and **(e-f)** leaf level reflectance values (Red at 680 nm
1179 and NIR at 800 nm), **(g-h)** leaf level vegetation indices (NDVI (680, 800 nm) and PRI (531, 570
1180 nm)). Samples include all 40 tree species surveyed at the Brazil site (grey circles); the subset of
1181 11 trees whose ages were precisely known and were the basis for the main part of this study
1182 (black squares) follow the same pattern as the larger community. Branch height (a proxy
1183 integrating effects of both light environment and gravitational component of leaf water potential)
1184 is generally a better predictor of leaf traits than branch depth (a proxy of light environment).

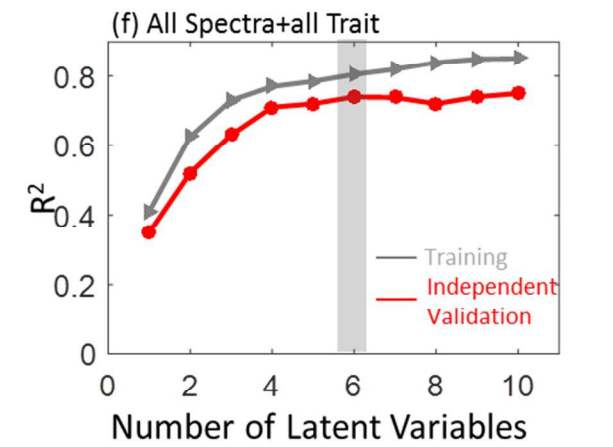
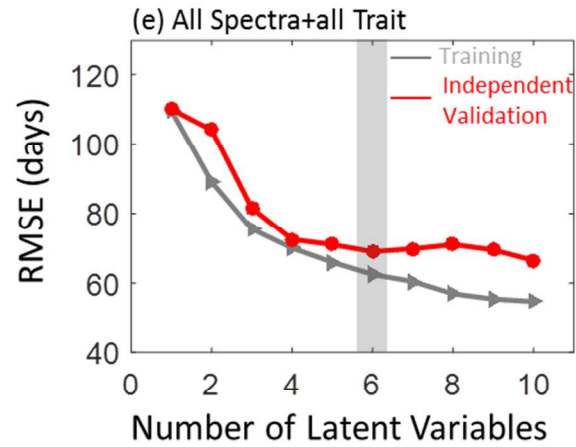
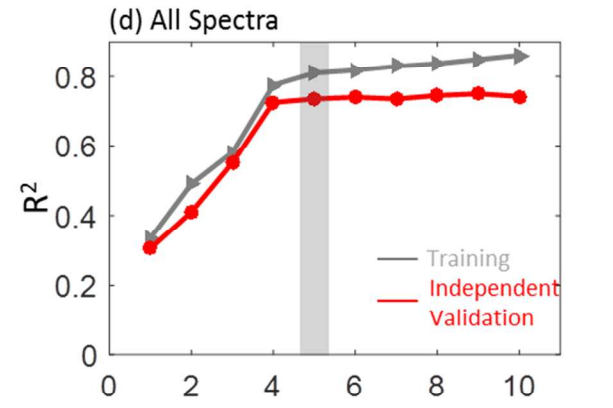
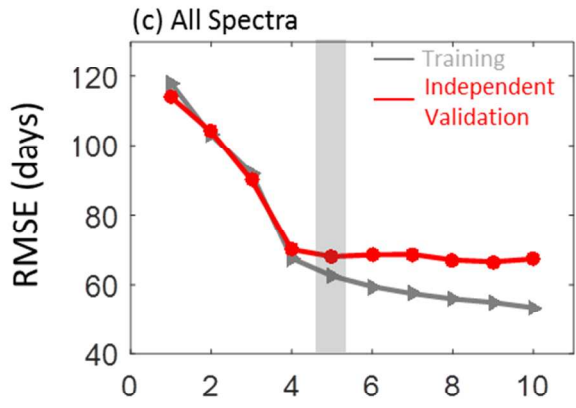
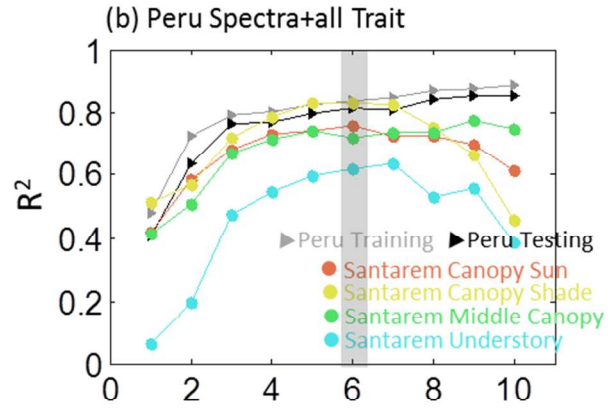
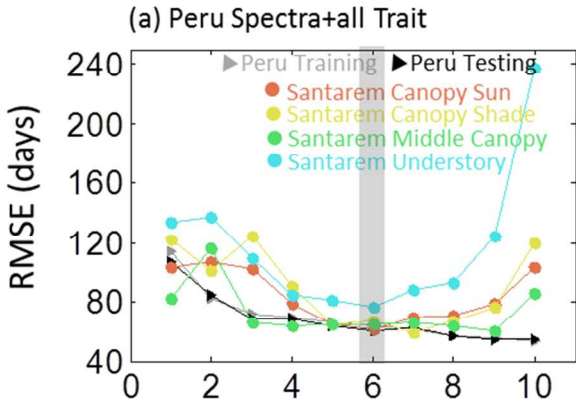
1185
1186

1187 **Figure S5.** Leaf age residuals (observed age – modeled age) plotted against observed age, where
1188 the "All Spectra" model was used here (optimal 5-variable model, Fig. 9d). **(a):** Peru data; **(b):**
1189 Brazil data. The grey circles indicate each individual leaf, and the black lines indicate the
1190 quadratic fitting curve.



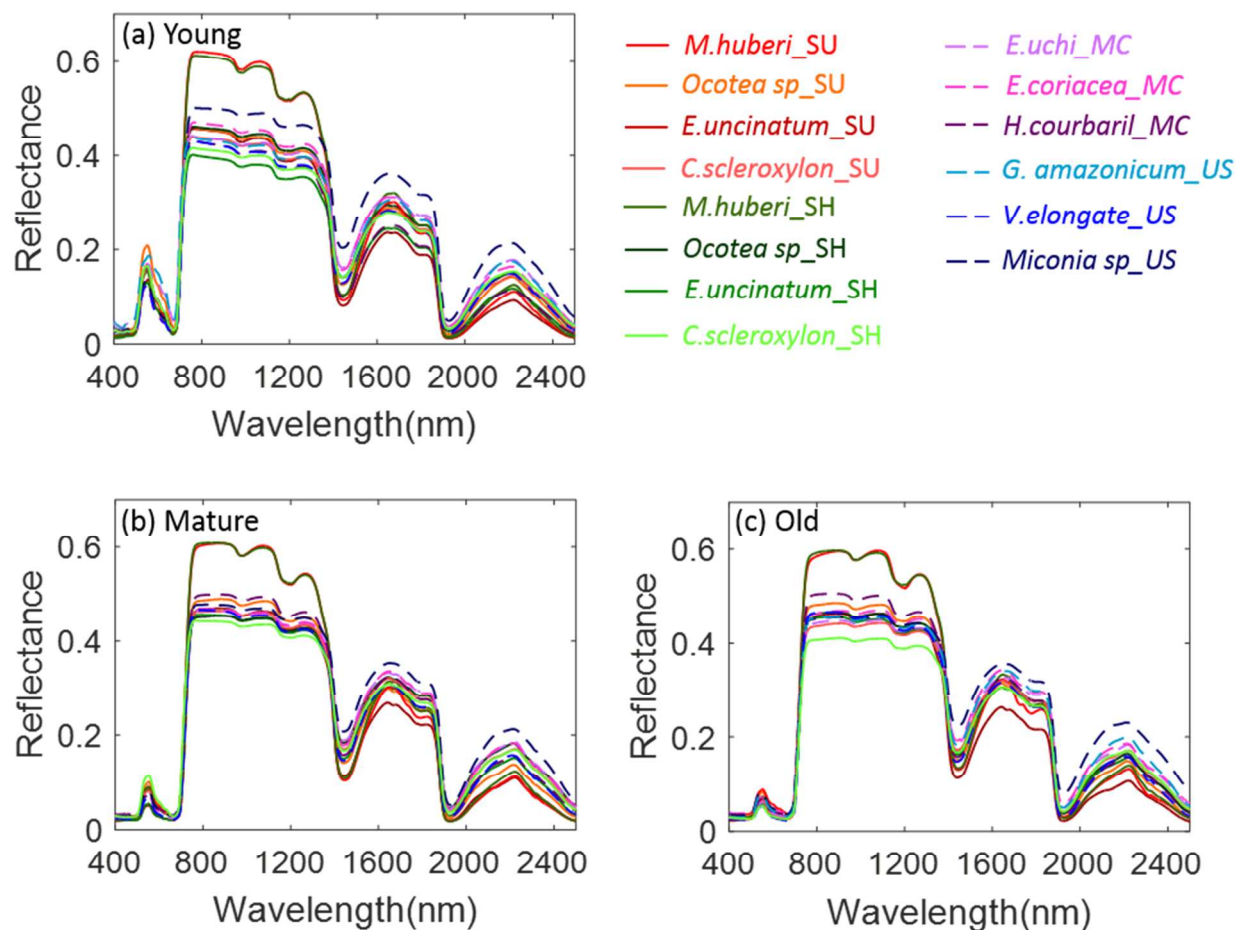
1191
1192
1193

1194 **Figure S6.** Metrics of fit for PLSR leaf age models versus number of latent variables included in
1195 the model for models shown in Fig. 9. **(a)** RMSE and **(b)** R^2 for the “Peru Spectra+all Trait”
1196 model; **(c)** RMSE and **(d)** R^2 for the “All Spectra” model; **(e)** RMSE and **(f)** R^2 for the “All
1197 Spectra+all Trait” model. Different symbols in (a) and (b) represent different datasets: Peru
1198 training data (grey triangles, n=12 trees); Peru testing data (black triangles, n=12); Independent
1199 data from the Brazil site, including canopy sun (red circles, n=4), canopy shade (yellow circles,
1200 n=4), mid-canopy (green circles, n=3), and understory (blue circles, n=4). Different color lines in
1201 (c), (d), (e) and (f) represent different datasets: grey lines—the training data (10 out of 12 trees
1202 from Peru, 3 out of 4 trees from Brazilian canopy sun, canopy shade, and understory,
1203 respectively, and 2 out of 3 trees from Brazilian mid-canopy); red lines—-independent validation
1204 data from the remaining data which were not used for model training. The grey shading in all
1205 panels indicates the optimum number of latent variables (minimizing RMSE and maximizing R^2
1206 for the independent dataset), including 6 latent variables for the “Peru Spectra+all Trait” model,
1207 5 latent variables for the “All Spectra” model, and 6 latent variables for the “All Spectra+all
1208 Trait” model.
1209



1210
1211

1212 **Figure S7.** Mean leaf level hyperspectral reflectance for three leaf age classes at the Brazil site:
 1213 **(a)** young (20-60 days), **(b)** mature (150-220 days), and **(c)** old (≥ 300 days) age classes.
 1214 Different tree-environment combinations are represented by different colored lines and ID codes
 1215 in the legend (indicated by a “*species name_canopy position*” label in the legend, where
 1216 “canopy position” is represented by four codes: ‘SU’ sunlit, ‘SH’ shaded, ‘MC’ mid-canopy, and
 1217 ‘US’ understory). Solid lines are for canopy leaves, and dashed lines for others.



1218
 1219
 1220
 1221

1222 **Table S1.** Summary statistics for leaf traits (LMA gm^{-2} and LWC %) and NIR reflectance (800
 1223 nm) under 4 canopy environments and across 2 forest sites. M=mean, SD=standard deviation.
 1224 Range=minimum and maximum values, and N=number of leaf samples.

Scenarios	Trait Type		Brazil	Brazil	Brazil Mid-	Brazil	Brazil	Peru
			Canopy Sun	Canopy Shade	Canopy	Understory	All	Canopy Sun
Entire leaf life span (7days- 400 days)	LMA	M(SD)	149.8 (50.7)	123.0 (58.4)	94.8 (21.4)	58.5 (11.7)	112 (53.6)	116 (37.7)
		Range	69.7-269.5	51.8-245.4	35.3-134.8	40.4-92.0	35.3-269.5	34.6-210.5
		N	150	139	122	93	504	1072
	LWC	M(SD)	52 (5)	55 (5)	53 (8)	66 (4)	56 (8)	56 (10)
		Range	42-68	43-72	42-83	59-79	42-83	34-81
		N	150	139	124	94	507	1072
	NIR	M(SD)	0.51 (0.07)	0.50 (0.08)	0.46 (0.03)	0.47 (0.04)	0.49 (0.06)	0.51 (0.06)
		Range	0.36-0.64	0.38-0.64	0.35-0.52	0.36-0.57	0.35-0.64	0.35-0.69
		N	224	207	186	142	759	1072

1225

1226

1227

1228 **Table S2.** Leaf age model performance assessment for Brazil data under four scenarios: the
 1229 “Peru Spectra” model (parameterized by using Peruvian leaf spectra only; five latent variables),
 1230 the “Peru Spectra+all Trait” model (parameterized by using Peruvian leaf spectra and traits; six
 1231 latent variables), the “All Spectra” model (parameterized by using both Brazilian and Peruvian
 1232 leaf spectra; five latent variables), and the “All Spectra+all Trait” model (parameterized by using
 1233 both Brazilian and Peruvian leaf spectra and traits; six latent variables). Three metrics are used to
 1234 measure the goodness of model fit, including coefficient of determination (R^2), root-mean-square
 1235 error (RMSE), and the Akaije Information Criterion (AIC; Akaike, 1974; Aho *et al.*, 2014). The
 1236 AIC was calculated by using the formula $AIC = N \times \log(\delta^2) + 2 \times m$, where N is the number of
 1237 leaves (summarized in Table S1), δ is RMSE (Fig. 9), and m is the optimum latent variable
 1238 number used for each PLSR modeling scenario (Fig. S6).

1239

Scenarios	Brazil Canopy Sun			Brazil Canopy Shade			Brazil Mid-Canopy			Brazil Understory			Brazil All		
	R^2	RMSE (days)	AIC	R^2	RMSE (days)	AIC	R^2	RMSE (days)	AIC	R^2	RMSE (days)	AIC	R^2	RMSE (days)	AIC
Peru Spectra	0.77	61.8	1857.5	0.77	62.4	1721.3	0.71	79.9	1639.6	0.47	89.8	1287.3	0.64	72.3	6508.3
All Spectra	0.76	75.1	1948.8	0.80	56.7	1682.0	0.77	78.1	1635.2	0.57	87.5	1283.9	0.69	73.7	6539.4
All Spectra	0.84	45.1	1716.4	0.89	48.2	1614.4	0.82	48.2	1451.6	0.73	71.7	1223.4	0.79	53.2	6042.6
All Spectra +all Trait	0.85	46.7	1736.0	0.88	48.7	1622.7	0.78	47.0	1446.3	0.82	60.0	1176.8	0.81	50.4	5962.5

1240