Supporting Information for Article: Leaf aging of Amazonian canopy trees as revealed by spectral and physiochemical measurements

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Supporting Information: Methods S1, 2 tables and 5 colour figures

Methods S1

Branch sampling

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

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

Leaf traits

We measured an important morphological trait – leaf mass per area (LMA). LMA is the ratio of the dry mass of a leaf to its surface area, it is intimately connected to the resource use economy of the plant (Reich *et al.,* 1997) as it can be understood as the leaf-level cost of light interception (Gutschick & Wiegel, 1988). LMA is, therefore, a key

trait indicative of plant physiological processes ranging from light capture (Niinemets *et al.*, 1999) to growth rates (Poorter *et al.*, 2009) as well as plant life strategies (Westoby *et al.*, 2002).

In terms of biochemical traits, we measured leaf water- (LWC), nitrogen- (N_{mass}), phosphorous- (P_{mass}) and carbon content (C_{mass}). LWC is a useful indicator of plant water balance, since it expresses the relative amount of water present on the plant tissues and it is intimately related to several leaf physiological variables, such as leaf turgor, growth, stomatal conductance, transpiration, photosynthesis and respiration (Kramer & Boyer, 1995). Nitrogen is an integral component to the proteins of the photosynthetic machinery responsible for drawdown of carbon dioxide (CO_2) inside the leaf while phosphorus, being a fundamental component of nucleic acids, lipid membranes and bioenergetic molecules such as ATP, is an essential nutrient in photosynthetic carbon assimilation and protein synthesis (Field & Mooney, 1986). Thus, both N_{mass} and P_{mass} have been directly linked to rates of photosynthetic activity in leaves (Raaimakers et al., 1995; Evans & Poorter, 2001; Reich et al., 2008, among others). Carbon is a major component in cellulose and lignin, which are used to build the cell walls of leaf tissues (Kokaly et al., 2009).

Leaf trait measurements

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

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

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

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

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

where: leaf fresh and dry mass are in grams (g), leaf area in square metres (m^{-2}) for LMA calculations and in square centimetres (cm^{-2}) for leaf volume calculations, leaf thickness in millimetres (mm) and leaf volume in cubic centimetres (cm^3).

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

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

Table S1. Leaf sampling scheme by tree, canopy position (SU = sun) and leaf age class. Leaf age classes: Y1, Y2, Y3 and young/mature (Y/M) are developing leaves collected after 1, 2, 3 and 4 wk of active leaf expansion, respectively; M, mature leaves; O, old leaves showing initial signs of senescence; S, senescent leaves in the process of dying and abscising.

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

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

| Vegetation index	Acronym	Equations	
Narrow band normalised difference vegetation index	NB NDVI	$\dfrac{\rho800 - \rho680}{\rho800 + \rho680}$	where $\rho800$= measured percent reflectance at 800 nm and $\rho680$= measured percent reflectance at 680 nm
Narrow band enhanced vegetation index 2	NB EVI2	$2.5 \times \dfrac{\rho800 - \rho680}{\rho800 + 2.4 \times \rho680 + 1}$	
Broad band normalised difference vegetation index	BB NDVI	$\dfrac{NIR - RED}{NIR + RED}$	where NIR= measured reflectance of MODIS NIR broadband (819 to 898 nm) using the MODIS filter response functions and RED= measured reflectance of MODIS RED broadband (614 to 682 nm) using the MODIS filter response functions.
Broad band enhanced vegetation index 2	BB EVI2	$2.5 \times \dfrac{NIR - RED}{NIR + 2.4 \times RED + 1}$	
Red edge normalised difference vegetation index	RE NDVI	$\dfrac{\rho750 - \rho705}{\rho750 + \rho705}$	where $\rho750$= measured percent reflectance at 750 nm and $\rho705$= measured percent reflectance at 705 nm
Red edge enhanced vegetation index 2	RE EVI2	$2.5 \times \dfrac{\rho750 - \rho705}{\rho750 + 2.4 \times \rho705 + 1}$	
Normalised difference water index	NDWI	$\dfrac{\rho857 - \rho1240}{\rho857 + \rho1240}$	where $\rho857$= measured percent reflectance at 857 nm and $\rho1240$= measured percent reflectance at 1240 nm
Photosynthetic reflectance index	PRI	$\dfrac{\rho531 - \rho570}{\rho531 + \rho570}$	where $\rho531$= measured percent reflectance at 531 nm and $\rho570$= measured percent reflectance at 570 nm
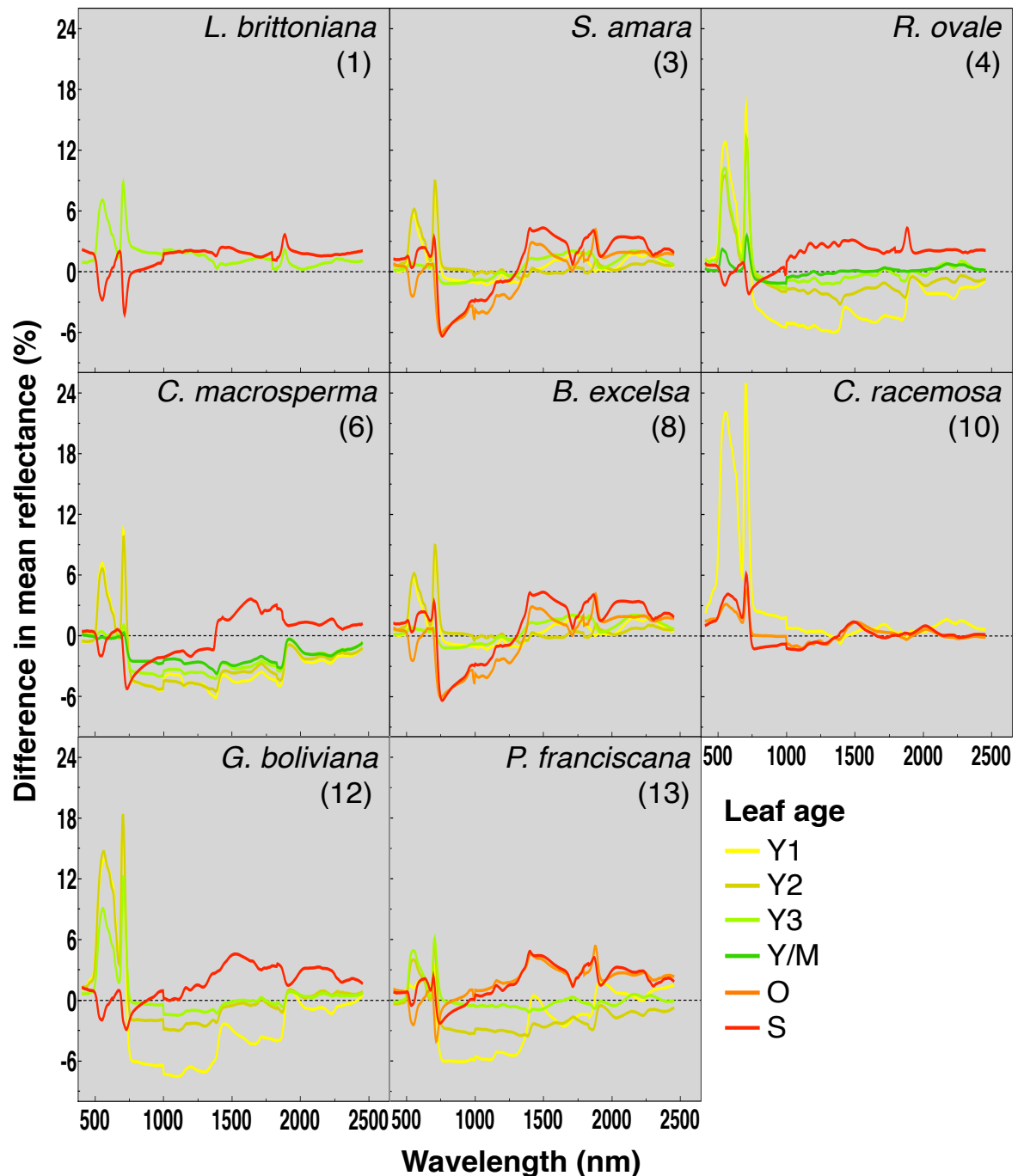
Fig. S1 Difference in mean reflectance between mature leaves and other leaf age classes for sun leaves of eight Amazonian canopy and emergent trees. Only trees (tree codes in brackets) with four or more sampled leaf age classes are included in this figure. Leaf age classes: Y1, Y2, Y3 and young/mature (Y/M) are developing leaves collected after 1, 2, 3 and 4 wk of active leaf expansion, respectively; M, mature leaves; O, old leaves showing initial signs of senescence; S, senescent leaves in the process of dying and abscising.
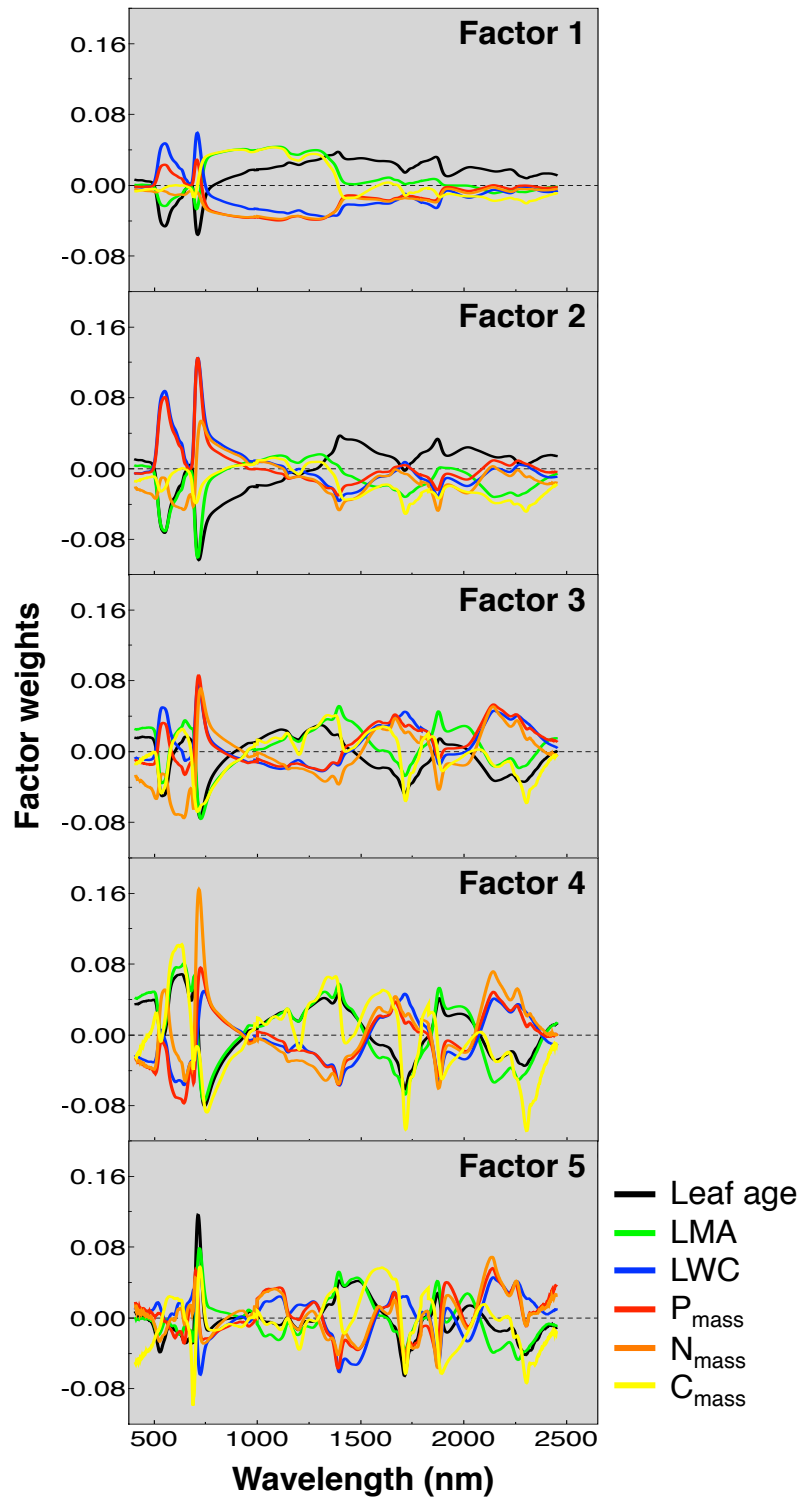
Fig. S2 Spectral weights (w*) for leaf age, leaf mass per area (LMA), leaf water content (LWC), phosphorous content (P_{mass}), nitrogen content (N_{mass}) and carbon content (C_{mass}). Only spectral weights for the first five PLSR latent factors (which cumulatively explain 83% of leaf age variation) are reported. Colour lines represent spectral weights for: leaf age (black), LMA (green), LWC (blue), P_{mass} (red), N_{mass} (orange) and C_{mass} (yellow).
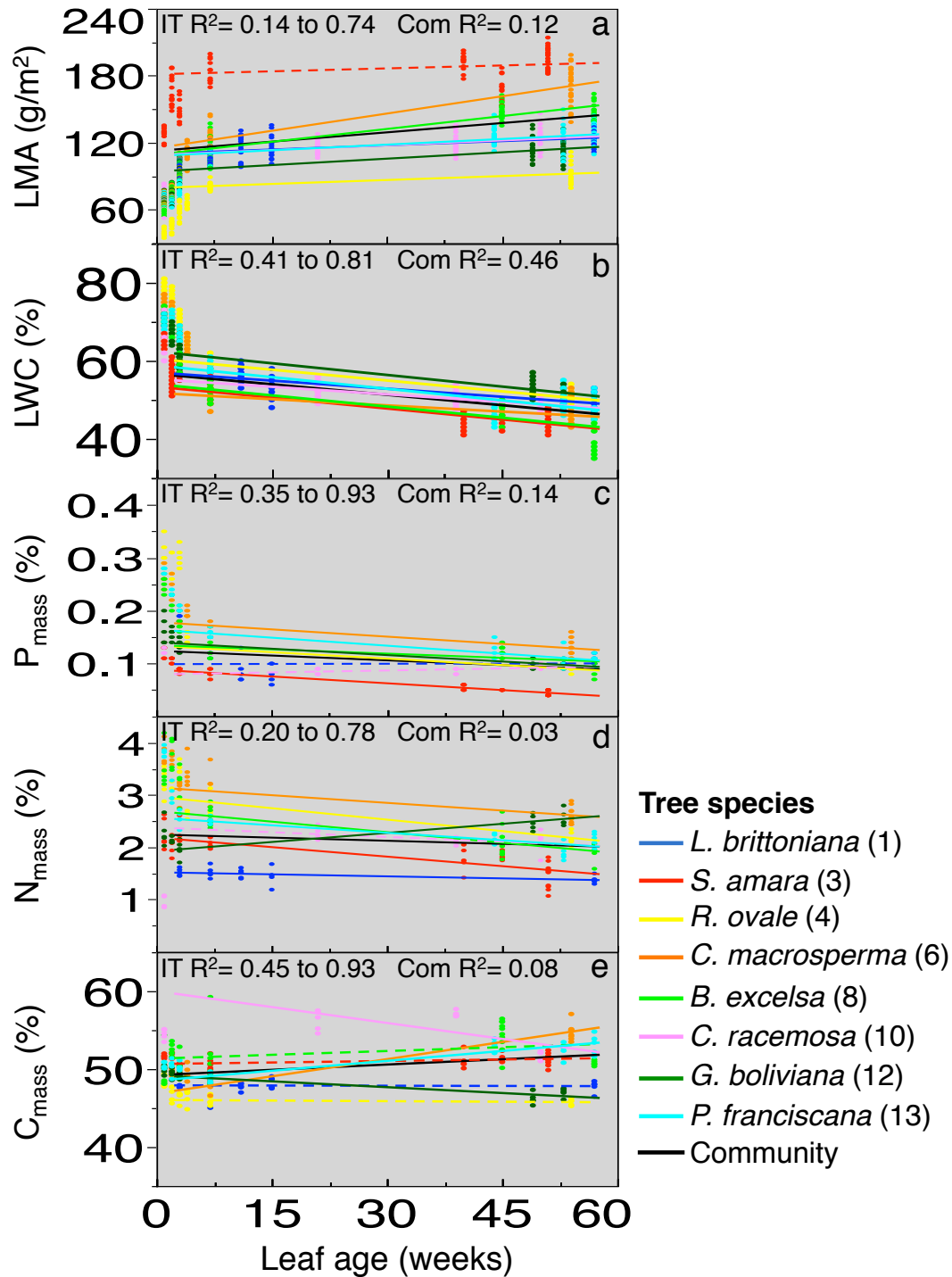
Fig. S3 Relationships between leaf traits and leaf age (not including developing leaves). Only trees (tree codes in brackets) with four or more sampled leaf age classes are included in these regression analyses. Individual leaf trait values are used as data points. Dashed lines are non-significant regressions. IT R^2 are for individual trees and Com R^2 are for all trees. All significant regression were $P <$ 0.05.
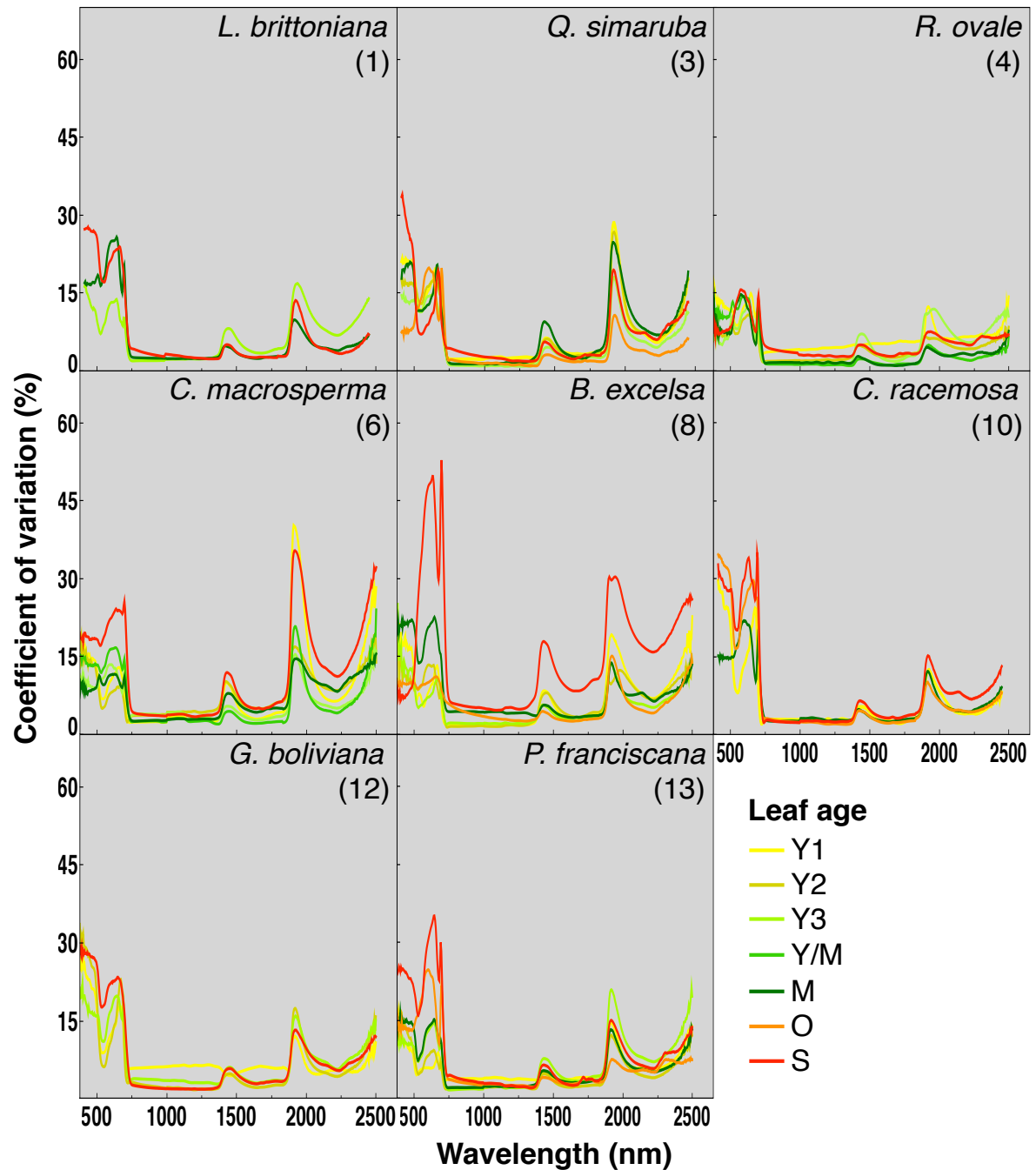
Fig. S4 Spectral coefficients of reflectance variation (CV) by leaf age class for sun leaves of eight Amazonian canopy and emergent trees. Only trees (tree codes in brackets) with four or more sampled leaf age classes are included in this figure. Leaf age classes: Y1, Y2, Y3 and young/mature (Y/M) are developing leaves collected after 1, 2, 3 and 4 wk of active leaf expansion, respectively; M, mature leaves; O, old leaves showing initial signs of senescence; S, senescent leaves in the process of dying and abscising.
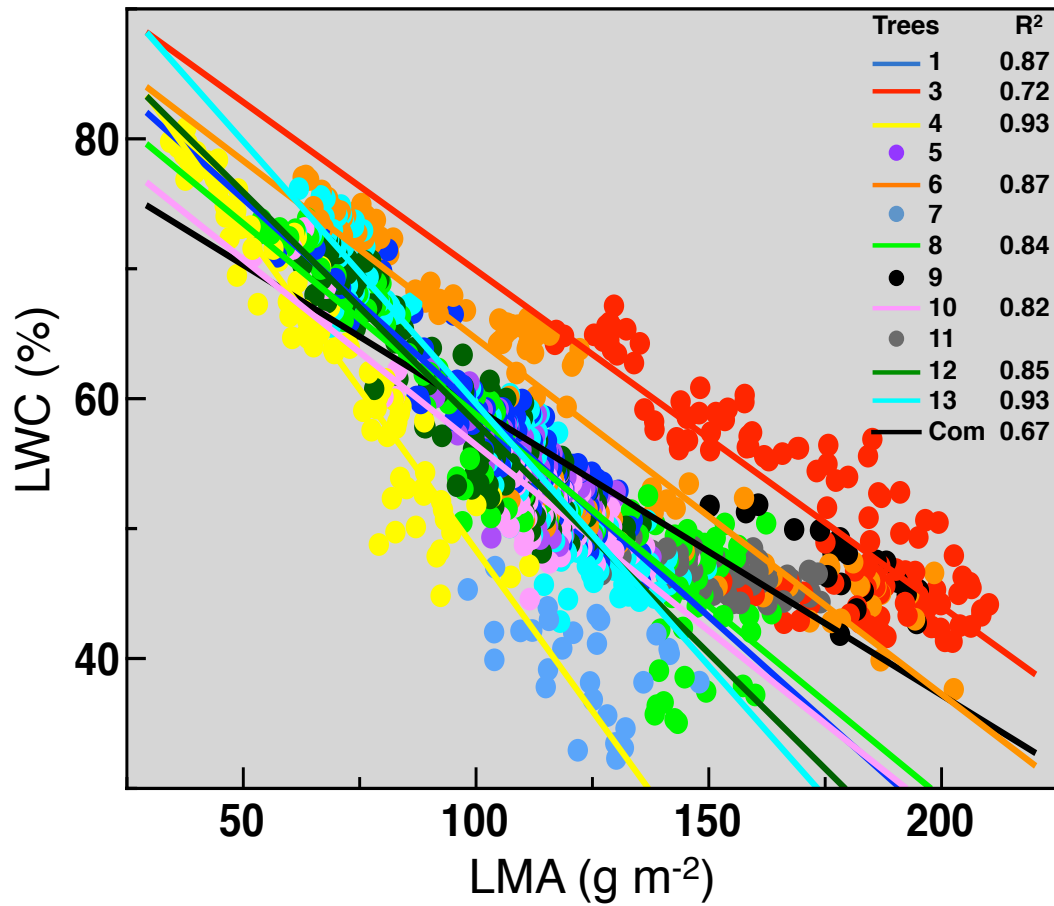
Fig. S5 Relationship between leaf water content (LWC) and leaf mass per area (LMA). Individual leaf trait values are used as data points. Only trees with four or more sampled leaf age classes are included in regression analyses. Solid lines represent significant regressions (all *P* < 0.0001) for individual trees (colour lines) and across all 12 trees (black line; Com).

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