New Phytologist Supporting Information Fig. S1, Tables S1–S4 and Methods S1 Article title: Logging and soil nutrients independently explain plant trait expression in tropical forests

Authors: Sabine Both, Terhi Riutta, C. E. Timothy Paine, Dafydd M. O. Elias, Rudi Chino, Annuar Jain, David Johnson, Ully H. Kritzler, Marianne Kuntz, Noreen Majalap-Lee , Nora Mielke, Milenka X. Montoya Pillco, Nicholas J. Ostle, Yit A. Teh, Yadvinder Malhi and David F. R. P. Burslem **Article acceptance date: 12 August 2018**

Methods S1 – Trait measurements

After collection, branches were placed in a bucket of water in the shade prior to processing, which was completed within 60 minutes. Before gas exchange measurements, branches were recut under water to prevent xylem embolism. Photosynthesis measurements were conducted using portable photosynthesis systems (Li-Cor 6400XT infrared gas analyser; Li-Cor BioSciences, Lincoln, NE, USA) and included light saturated photosynthetic rate (A_{sat}), maximum photosynthetic rate under high light and high carbon dioxide (A_{max}) and dark respiration (R_d). Measurements were made on mature leaves attached to the branch and were conducted between 9 am – 1 pm in order to avoid stomatal closure during daily temperature peaks.

Leaves and branches were stored with wet tissue paper in zip lock bags in cool boxes and transported to a field laboratory where they were measured for leaf fresh weight, leaf lamina thickness (L_{th}), and oven-dried leaf weight (after 72 h at 60 °C). Leaf force to punch (F_p, N mm^{-1}) was determined with a Chatillon punchometer and by dividing the observed force (N) required to puncture the leaf lamina by the circumference of the instrument's rod. Leaf area (LA) was measured by scanning leaves per tree (Canon LiDE 220 portable leaf scanner, Canon Inc., Tokyo, Japan) and determining leaf area by image analyses using ImageJ software (Schneider *et al.* 2012). Specific leaf area (SLA), leaf dry matter content (LDMC) and specific force to punch (F_p divided by lamina thickness, N mm^{-2}) were calculated from these measurements. Specific branch wood density was measured on 4-5 cm long branch segments of 2 – 4 cm diameter with and without bark using the water displacement method. Due to very similar results we report on branch samples with bark.

From each branch a collection of well-developed, mature leaves lacking herbivory were carefully cleaned of epiphylls, bulked and dried at 60 °C for 72 h. Sub-samples were finely ground and analysed for concentrations of total C, N, $\delta^{13}C$ and $\delta^{15}N$ using isotope ratio mass spectrometry (NCS 2500, CE Instruments, UK). Additional sub-samples were ground and digested using sulphuric acid and hydrogen peroxide. Concentrations of total P in the digests were measured using a flow injection auto-analyser (FIAstar™ 5000, Foss Tecator, Denmark) and the base cations Ca, K and Mg were measured with atomic absorption spectroscopy (AAS, Perkin Elmer AAnalyst 100, MA, USA). Further sub-samples were coarsely ground and analysed for concentrations of cellulose, hemicellulose, and lignin by sequential digestion of fibres using an ANKOM Fiber Analyzer (ANKOM Technology, Macedon, NY, USA).

Additional chemical analyses were conducted on 0.7 cm diameter leaf discs punched from fresh leaves immediately after collection and immersed in liquid N in a dry shipper transported to the field laboratory. These dry shippers were transported within 7 days to a central laboratory where the discs were stored at -80 °C until further processing. Concentrations of chlorophyll a, chlorophyll b and bulk carotenoids in the discs was conducted on methanol extracts using a UV spectrophotometer (Shimadzu UV-1800) measuring absorbance at 470, 662, 645, and 710 nm following the equations of Lichtenthaler & Buschmann (2001). Additional leaf discs were analysed for bulk phenol and tannin concentrations with a Folin-Ciocalteau Assay. Protocols for fibre, pigment, phenol and tannin analyses replicate those of the Carnegie Airborne Observatory Spectronomics laboratory (https://cao.carnegiescience.edu/spectranomics-protocols).

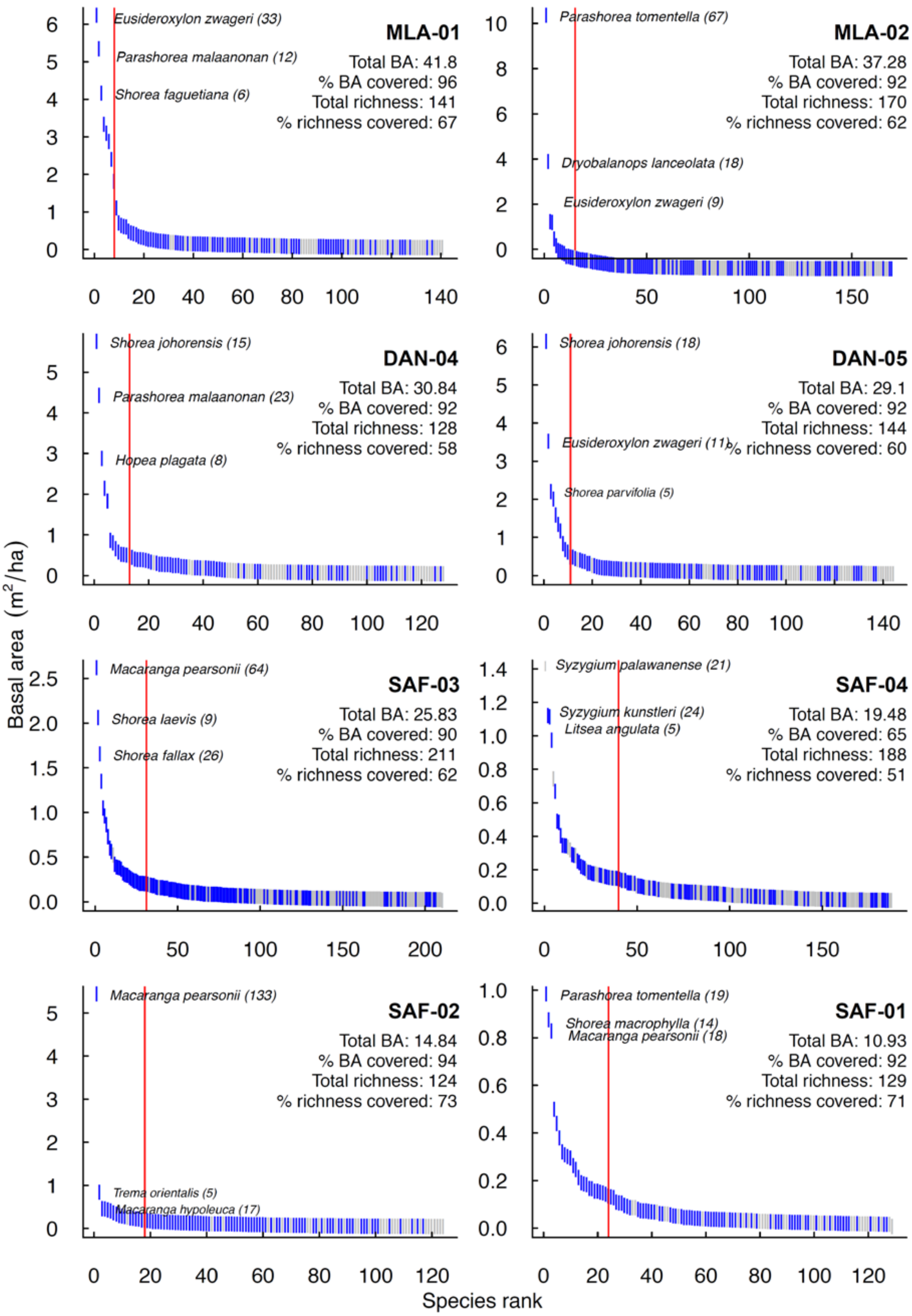

Figure S1: Realized coverage of species measured across the study plots. Species are ranked by their contribution to basal area. Species with traits sampled (in any plot) are indicated in blue. The three species with highest contribution to plot basal area are named, numbers in parentheses show total number of individuals occurring in the respective plot. Red line indicates 70 % basal area threshold.

Table S1: Overview of functional traits measured in this study and numbers of replicates per individual branch. For more details on functions see Pérez-Harguindeguy *et al.* (2013).

Functional traits	**Function**	**# Replicates**
Ca_m	Regulator for growth processes and responses to environmental stresses, including stomatal function, expressed per unit of dry leaf mass [$mg \cdot g^{-1}$]	1 bulk sample
Mg_m	Facilitates function of many cellular enzymes, enables light absorbance in chlorophyll, expressed per unit of dry leaf mass [$mg \cdot g^{-1}$]	1 bulk sample
K_m	Regulating role for stomata conductance, expressed per unit of dry leaf mass [$mg \cdot g^{-1}$]	1 bulk sample
N_m, N_a	Close correlation with maximum photosynthetic rate, proxy of nutrient quality for herbivores, expressed as total concentration [%] or per unit leaf area [$mg \cdot mm^{-2}$]	1 bulk sample
P_m, P_a	Close correlation with maximum photosynthetic rate, proxy of nutrient quality for herbivores, expressed per unit of dry leaf mass [$mg \cdot g^{-1}$] or per unit leaf area [$mg \cdot mm^{-2}$]	1 bulk sample
C_m	Relates to resource capture and defence, expressed as total concentration [%]	1 bulk sample
$\delta^{13}C$	Indicator of leaf-level water-use efficiency, expressed relative to Vienna Pee Dee Belemnite (VPDB) as $\delta^{13}C$ in units of per mil [‰]	1 bulk sample
$\delta^{15}N$	Indicator for nitrogen acquisition via symbiotic fungi and microbes [‰]	1 bulk sample
Dark respiration (R_d)	Measure of basal metabolism, proxy for average realised night-time respiratory carbon flux. R_d scales with other metabolic, structural and chemical aspects of the leaf economic spectrum [$\mu mol\ CO_2\ m^{-2} \cdot s^{-1}$]	3 leaves
Maximum photosynthetic rate (A_{max})	Maximum photosynthetic capacity, measure of the maximum rate at which leaves are able to fix carbon during photosynthesis [$\mu mol\ CO_2\ m^{-2} \cdot s^{-1}$]	3 leaves
Light-saturated photosynthetic rate (A_{sat})	Light-saturated net photosynthesis, measure for metabolic capacity at ambient CO_2 concentrations. Proxy for productivity and growth and scales with other structural, chemical and longevity aspects of the leaf economic spectrum [$\mu mol\ CO_2\ m^{-2} \cdot s^{-1}$]	3 leaves
Pigments (mass-based: chlorophyll a_m, chlorophyll b_m, $carotenoids_m$; area-based: chlorophyll a_a, chlorophyll b_a, $carotenoids_a$,)	Play crucial role for photosynthesis, facilitate light-harvesting capacity, expressed per unit of dry leaf mass [$mg\ g^{-1}$] or per unit of leaf area [$mg\ mm^{-2}$]	1 bulk sample of frozen leaf discs
Specific leaf area (SLA)	One-sided area of a fresh leaf including petiole, divided by its oven-dry mass. Proxy for growth and photosynthesis, indicator for carbon investment and leaf longevity [$mm^2 \cdot mg^{-1}$]	8 leaves
Leaf area	Measure of leaf size including petiole, important for light interception and temperature regulation [mm^2]	8 leaves

Table S1 *(continued)*

Functional traits	**Function**	**# Replicates**
Leaf fresh weight, leaf dry weight	Proxy of leaf size, indicator of water content [mg]	8 leaves
Leaf thickness	Indicator of physical strength of leaf, linked to the number and thickness of mesophyll layers [mm]	3 leaves
Leaf force to punch	Physical strength of leaf, indicator for prolonged leaf life span [$N \cdot mm^{-1}$]	5 leaves
Specific leaf force to punch	Force to punch divided by lamina thickness, i.e. toughness expressed per unit leaf thickness [$N \cdot mm^{-2}$]	5 leaves
Leaf dry matter content (LDMC)	Oven-dry leaf mass divided by its water-saturated fresh mass, indicator of leaf toughness and leaf lifespan [$mg \cdot g^{-1}$]	8 leaves
Total phenol concentration	Contribution to plant defence, related to leaf longevity, expressed related to leaf dry mass [$mg\ g^{-1}$]	1 bulk sample of frozen leaf discs
Total tannin concentration	Contribution to plant defence, related to leaf longevity, expressed per unit of dry leaf mass [$mg\ g^{-1}$]	1 bulk sample of frozen leaf discs
Leaf fibres ((hemi-)cellulose, lignin)	Contribution to physical strength and stability of plant cells, , expressed as total concentration [%]	1 bulk sample
Branch specific density	Indicator of stability, defence, architecture, hydraulics, carbon gain and growth potential of plants, oven-dry mass of a branch section divided by the fresh volume of the same section [$g \cdot cm^{-3}$]	6 branch segments, of which 3 with and 3 without bark

55

Table S2: Loadings (in %) of the soil environmental variables in the Principal Component Analysis (Fig. 1). Loadings in bold show the three highest loading variables on that PC axis. Shown are the first four principal components for which the eigenvalues are > 1.

soil properties	**PC1**	**PC2**	**PC3**	**PC4**
eCEC [mmol$^+$ kg^{-1}]	12.62	3.24	2.15	3.33
Total Mg [mg kg^{-1}]	12.29	4.89	0.93	4.27
Total P [mg kg^{-1}]	12.17	3.27	0.34	9.71
pH (H_2O)	11.86	4.90	6.11	3.64
Total Ca [mg kg^{-1}]	11.28	3.56	8.83	5.37
Exchangable Mg [µg 10 cm^2 14 days^{-1}]	7.27	3.16	8.54	20.07
Exchangable Ca [µg 10 cm^2 14 days^{-1}]	6.71	11.07	2.24	5.18
Total K [mg kg^{-1}]	6.55	9.59	10.43	6.60
C [%]	6.24	10.73	7.13	3.78
Exchangable K [µg 10 cm^2 14 days^{-1}]	4.38	12.04	1.93	3.25
NO_3^- [µg 10 cm^2 14 days^{-1}]	2.95	6.93	13.92	5.48
Extractable P [µg 10 cm^2 14 days^{-1}]	2.52	4.55	15.76	18.32
NH_4^+ [µg 10 cm^2 14 days^{-1}]	2.02	10.94	9.20	6.52
N [%]	1.16	11.13	12.50	4.46

Table S3: Loadings (in %) of the CWM traits in the Principal Component Analysis (Fig. 2). Loadings in bold show the five highest loading variables on that PC axis. Shown are the first five principal components for which the eigenvalues are > 1.

CWM trait	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Chl b_m [mg g^{-1}]	**4.44**	0.30	0.95	0.63	2.82
N_a [mg mm^{-2}]	**4.37**	0.18	2.70	1.90	0.49
Branch density [g cm^{-3}]	**4.25**	2.14	0.73	1.13	2.84
A_{sat} [µmol CO_2 m^{-2} s^{-1}]	**4.25**	1.09	2.97	1.59	2.91
A_{max} [µmol CO_2 m^{-2} s^{-1}]	**4.21**	1.42	3.27	1.80	2.46
SLA [mm^2 mg^{-1}]	4.17	2.55	1.98	1.23	1.13
Specific force to punch [N mm^{-2}]	4.12	1.19	2.33	4.52	1.16
$Carotenoids_a$ [mg mm^{-2}]	4.01	2.35	3.87	2.02	0.61
Leaf thickness [mm]	3.97	0.20	2.01	4.97	2.56
Total P_a [mg mm^{-2}]	3.97	3.29	2.32	0.24	0.20
$\delta^{13}C$ [‰]	3.95	3.22	2.50	0.17	2.21
Chl a_a [mg mm^{-2}]	3.90	2.45	4.09	2.84	0.88
Chl a_m [mg g^{-1}]	3.82	2.34	4.03	0.30	2.47
R_d [µmol CO_2 m^{-2} s^{-1}]	3.69	2.59	4.66	1.68	0.09
$Carotenoids_m$ [mg g^{-1}]	3.55	2.80	**5.41**	2.32	3.85
Force to punch [N mm^{-1}]	3.45	1.41	1.68	**8.04**	3.09
N_m [%]	3.43	4.33	2.57	1.90	0.85
Total phenol [mg g^{-1}]	3.40	3.51	1.01	6.01	3.72
$\delta^{15}N$ [‰]	3.36	0.88	3.06	**7.71**	4.69
Total K [mg g^{-1}]	3.03	2.71	3.42	**7.25**	0.99
Cellulose [%]	2.98	1.65	**6.28**	**6.85**	3.38
Chl b_a [mg mm^{-2}]	2.89	4.38	3.97	5.11	3.41
Lignin & recalcitrants [%]	2.65	4.73	2.79	**6.37**	1.03
Hemicellulose [%]	2.45	3.52	**7.49**	2.55	**7.51**
Total tannin [mg g^{-1}]	2.32	**6.22**	2.88	0.09	1.78
Leaf dry weight [mg]	2.31	**5.75**	0.60	4.66	2.56
Total Ca [mg g^{-1}]	2.25	4.89	3.37	2.04	**11.09**
LDMC [mg g^{-1}]	1.99	2.34	**8.31**	5.90	**6.71**
C [%]	1.35	**5.88**	1.65	1.77	**10.93**
Total P_m [mg g^{-1}]	0.58	**7.45**	0.22	1.26	1.14
LA [mm^2]	0.50	**7.02**	0.58	3.71	3.05
Total Mg [mg g^{-1}]	0.41	5.23	**6.32**	1.44	**7.38**

Table S4: Results from linear regression models underlying Fig. 3. Shown are proportions of variance explained by each predictor (in %) and significance levels indicated with * ($p < 0.05$) after correcting the p-values by using the false discovery rate method.

	Forest type	**Soil PC1**	**Soil PC2**	**Unexplained**
Rao's Q	1.3	2.5	42.0	54.2
Nutrients				
$\delta^{15}N$ [‰]	54.0	1.3	3.6	41.0
Total Ca [mg g^{-1}]	11.8	68.7	5.8	13.7
Total Mg [mg g^{-1}]	8.2	23.1	7.0	61.6
Total K [mg g^{-1}]	10.5	2.1	37.4	50.0
N_m [%]	41.1	20.8	22.4	15.7
Total P_m [mg g^{-1}]	4.1	72.5	2.4	20.9
C [%]	5.9	90.2 *	0.2	3.8
Photosynthesis				
$\delta^{13}C$ [‰]	56.1	9.3	27.1	7.6
Total P_a [mg mm^{-2}]	58.7	12.1	22.6	6.7
N_a [mg mm^{-2}]	73.3 *	2.2	21.3	3.3
R_d [µmol CO_2 m^{-2} s^{-1}]	39.4	4.0	35.6	21.0
A_{max} [µmol CO_2 m^{-2} s^{-1}]	68.2	1.6	17.8	12.4
A_{sat} [µmol CO_2 m^{-2} s^{-1}]	64.5	1.5	20.2	13.8
$Carotenoids_a$ [mg mm^{-2}]	66.2	10.3	17.0	6.5
$Carotenoids_m$ [mg g^{-1}]	41.8	21.8	3.5	32.9
Chl b_a [mg mm^{-2}]	33.5	21.8	12.8	31.8
Chl b_m [mg g^{-1}]	78.6	4.0	8.8	8.5
Chl a_a [mg mm^{-2}]	58.3	11.0	21.1	9.6
Chl a_m [mg g^{-1}]	64.3	13.5	0.9	21.2
SLA [mm^2 mg^{-1}]	68.5	13.6	11.6	6.2
Structure				
LA [mm^2]	5.3	63.1	10.2	21.4
Leaf dry weight [mg]	13.7	38.2	30.6	17.5
Leaf thickness [mm]	48.6	1.1	21.1	29.1
Specific force to punch [N mm^{-2}	74.6	1.8	1.0	22.7
Force to punch [N mm^{-1}]	64.4	1.6	1.1	32.9
LDMC [mg g^{-1}]	4.2	5.7	2.2	87.8
Branch density [g cm^{-3}]	67.4	5.3	10.1	17.2
Total phenol [mg g^{-1}]	63.1	22.6	4.7	9.6
Total tannin [mg g^{-1}]	30.6	50.0	0.3	19.1
Lignin & recalcitrants [%]	34.3	24.2	2.0	39.5
Cellulose [%]	43.0	2.0	8.8	46.2
Hemicellulose [%]	21.1	16.6	3.2	59.1

References

Lichtenthaler, H.K. and Buschmann, C. (2001) Current protocols in Food and Analytical Chemistry F4.3.8-F4.2.4

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., *et al.* (2013). New Handbook for standardized measurement of plant functional traits worldwide. *Aust. J. Bot.*, 61, 167–234

Schneider, C. A, Rasband, W.S. & Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nat. Methods, 9, 671–675