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Supplementary Information

Trait Estimation in Herbaceous Plant Assemblages from *in situ* Canopy Spectra. *Remote Sens.* 2013, *5*, 6323–6345

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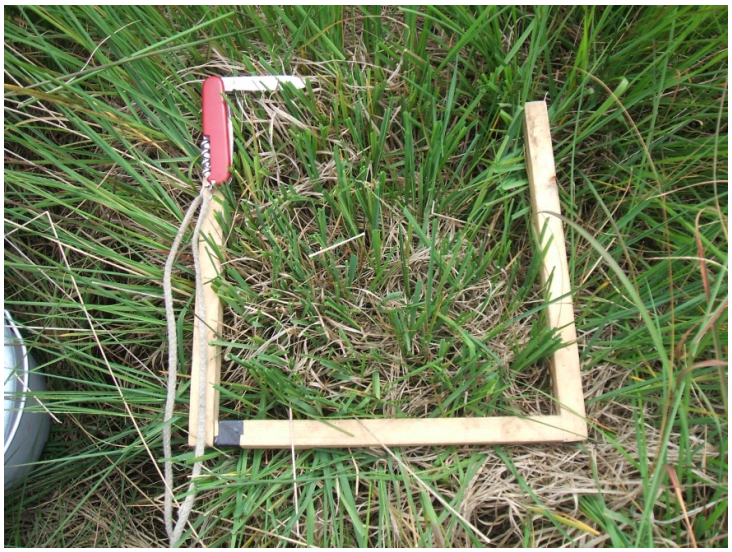
This supplementary material supports the main text with following:

Supplementary Data 1: Impression of Sampling and Plot Surveying

Figure S1. Picture of a typical vegetation plot. The pole indicating the lower left corner is seen bottom right. From there, the plot measured 2 × 2 m.

Figure S2. Biomass sampling. The 25 × 25 cm sampling frame is seen, just after the material has been cut.



Supplementary Data 2: Trait Determination Protocols and Summary Statistics

Subsample A was measured for fresh weight (f.w. (g)) directly after shredding and stored in a dry paper bag. Upon arrival at the laboratory facilities, the subsample was oven dried at 60 °C for 48 h and again weighed so that dry weight (d.w. (g)) and, subsequently, Specific Water Content (g water·g d.w.$^{-1}$) could be determined according to Equation 1.

$$SWC_{mass} = (f.w. - d.w.) / d.w. \quad (1)$$

To test the homogeneity of the subsampling scheme in general, and of subsample A in particular, three subsamples (A1, A2 and A3) were extracted and grounded to powder using a ball mill. Leaf Nitrogen and Leaf Carbon Concentration (LNC_{mass}, LCC_{mass}, mg·g^{-1}) were determined by dry combustion with a Flash EA112 element analyzer (Thermo Scientific, Rodana, Italy) for A1–A3, resulting in 3 *·40= 120 LNC_{mass} and LCC_{mass} measurements. Subsequently, the coefficient of variation of the LNC_{mass} and LPC_{mass} values of each plot was calculated. These values were low: mean CV LNC_{mass}: 4.98 ± 1.35 (95% confidence interval, n = 40) and for LCC_{mass}: 0.94 ± 0.25 (95% confidence interval, n = 40). In all, this demonstrated for us that at least subsample A, and therefore likely also subsamples B and C, was internally homogeneous, which allowed us to proceed with taking samples for analysis from each subsample A–C.

Approximately 50 mg ground material of subsample A (from either A1–A3, or the remaining material of subsample A) was digested in 4:1 HNO_3 − HCl mixture, after which Leaf Phosphorus Concentration (LPC_{mass}, mg·g^{-1}) was measured colorimetrically on a spectrophotometer (UV-1601 PC, Shimadzu Corporation, Tokyo, Japan) [1].

$Lignin_{mass}$ was determined following [2]. In short, 250 mg dried and ground material was sequentially extracted in H_2O, 80% MeOH and $CHCl_3$, followed by hydrolysis in 3M HCl and warming in a muffle oven at 500 °C for 5.5 h. The remaining sample material contained only lignin and cellulose. These concentrations were calculated by (1) C and N measurements in the residue

(following the same protocol mentioned above) and (2) the difference in C concentration between cellulose and lignin.

The second subsample (subsample B) was wrapped in moist tissues, sealed in a plastic bag and kept refrigerated until arrival at the laboratory where they were stored in a freezer. Subsequently, the samples were freeze-dried. Total phenol concentration ($mg \cdot g^{-1}$) was measured with the FolinCiocalteau method after extraction in 50% MeOH, using tannic acid (Merck, Darmstadt, Germany) as a standard. The tannin fraction of the extract was precipitated with PVPP, after which remaining simple phenol were measured as above. $Tannin_{mass}$ was determined as the difference between the total $phenol_{mass}$ concentration and the simple phenol concentration [3].

Subsample C was used to determine Chl a, Chl b and total Chl according to [4]. To prevent Chl disintegration by sunlight and warmth, the sample was wrapped in moist paper towels and tin foil, sealed in a plastic bag and stored on dry ice for immediate freezing and stored in freezer upon arrival at the laboratory. During the Chl determination procedure, the samples were kept on ice as much as possible and the extraction was performed in a dark and refrigerated room. Chl was extracted in a 100% MeOH solution (we used MeOH instead 80% acetone or DMF, because acetone does not completely extract Chl b [4] while DMF was considered too hazardous for convenient use) and full range absorbance was measured with a spectrometer between 640 and 750 nm. Chl a and Chl b were simultaneously derived from the same extract. This is possible because the wavelengths where maximum absorbance occurs deviate between the two Chl variants. The exact values of the maximum absorbance wavelengths vary with various sources [5]. We manually determined the peak absorbance of Chl a and fixed the peak absorbance of Chl b at 13.2 nm lower. Employing given extinction coefficients for Chl a and Chl b [4], Chl_{mass} could be calculated.

Subsample D was collected on the site and consisted of 4–5 intact and mature leaves that were free from herbivore activity and disease, from the 3–4 most dominant species of the plot. We specifically collected a sample of leaves that was representative for the plots' floristic composition. Note that is in contrast to the protocol of [6] that specifically instructs to collect sunlit leaves only. In case of *C. vulgaris* and *E. tetralix*, leaves were stripped from young branches. The sample was wrapped in moist paper tissues and stored in a plastic bag in a cooling box. The leaves were scanned on a flatbed scanner the same day, oven dried at 60°C for 48 h and finally weighed to acquire the sample dry weight (mg). From the scanned images, the one-sided leaf area could be determined by referring to the area of simultaneously scanned reference object. This was done in ImageJ [7]. For the cylindrical *J. effusus* leaves, we felt that this procedure yielded the projected area (height (h) × diameter (d)) rather than the actual one-sided area, which would be given by $A = 0.5 \times \pi \times d \times h$. Therefore, we could obtain the actual one sided area by multiplying the original area estimate ($d \times h$) with $0.5 \times \pi$. The specific leaf area (SLA) was calculated according to Equation (2).

$$SLA\ (mm^2 \cdot mg^{-1}) = area\ (mm^2)/dry\ weight\ (mg) \quad (2)$$

Table S1. Summary statistics of trait values expressed on three different levels.

	Mass Traits					Leaf Surface Traits					Canopy Surface Traits				
	min	max	mean	median	sd	min	max	mean	median	sd	min	max	mean	median	sd
LNC	9.23	26.80	14.78	13.65	4.73	279.98	2284.26	945.65	889.40	441.83	523.55	7720.81	2955.20	2668.75	1673.10
LPC	0.37	3.69	1.38	1.06	0.91	29.01	294.57	78.33	60.04	51.32	52.22	995.65	261.09	176.48	221.89
LCC	406.97	505.70	447.90	441.20	25.50	10893.28	68501.06	29561.15	27538.19	12938.31	20370.43	231533.59	92739.85	87995.13	50706.35
Chl a	0.25	3.74	1.30	1.26	0.64	18.25	335.54	82.66	70.96	55.55	47.22	808.66	260.82	197.17	182.74
Chl b	0.22	4.05	1.70	1.75	0.77	17.02	362.79	107.21	94.32	66.60	54.30	920.84	337.15	272.85	220.71
Chl tot	0.34	5.68	2.23	2.30	1.02	26.04	509.14	141.11	120.50	89.20	83.06	1227.03	444.24	351.71	295.20
Lignin	36.66	220.00	113.16	101.49	49.58	1909.95	20254.89	7984.87	5875.82	5579.10	3420.99	65423.30	23042.84	21904.07	14598.81
Phenol	0.02	0.27	0.07	0.05	0.05	0.77	21.23	5.31	2.94	5.10	1.47	67.72	14.67	8.68	13.67
Tannin	0.06	0.80	0.31	0.21	0.23	2.07	71.81	22.33	12.97	21.30	3.86	210.16	58.60	38.21	49.38
SWC	1.02	4.91	2.03	1.91	0.82	49.26	271.51	122.93	114.36	49.2	92.12	1083.01	395.68	296.36	235.43

Figure S3. Scatterplots and correlation coefficients for all trait pairs, expressed on mass basis, as well as for SLA and LAI. Correlations significant at $p<0.05$ are indicated with an asterisk.

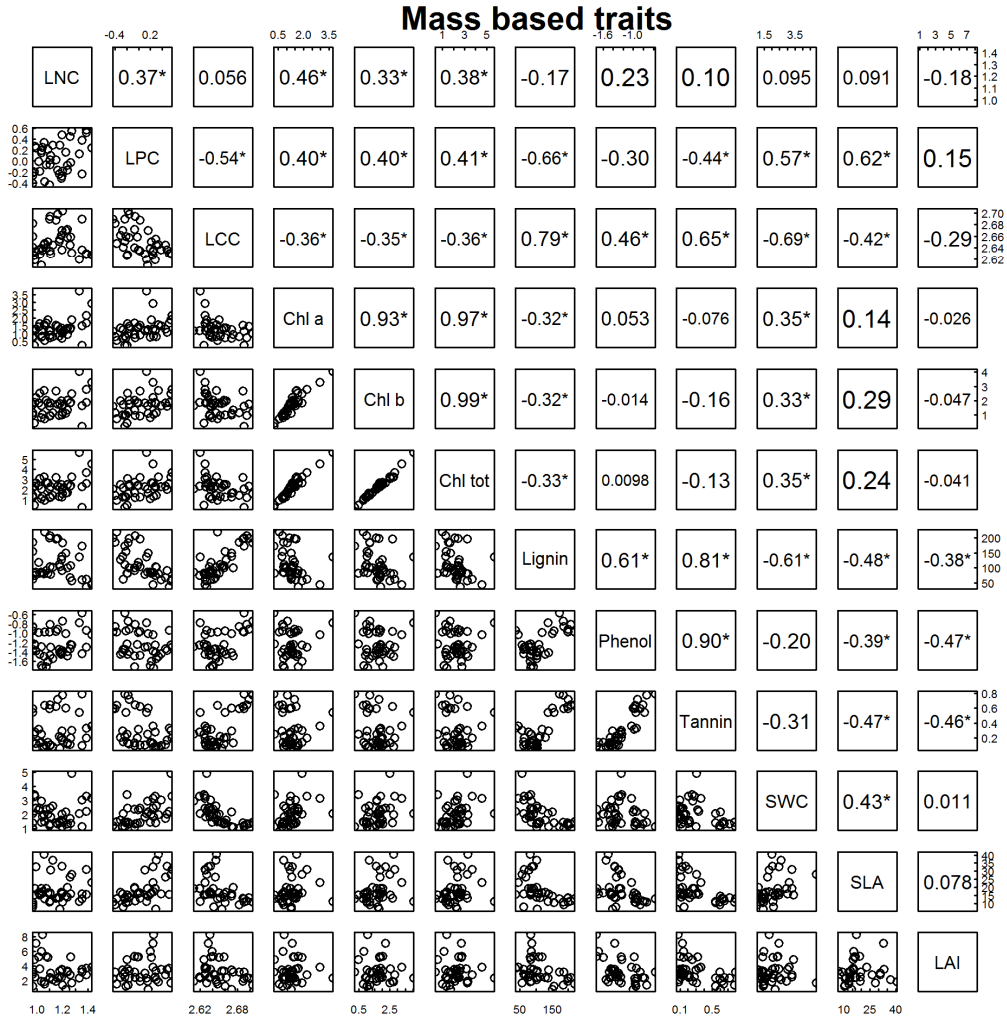


Figure S4. Scatterplots and correlation coefficients for all trait pairs, expressed on leaf surface basis, as well as for SLA and LAI. Correlations significant at $p<0.05$ are indicated with an asterisk.

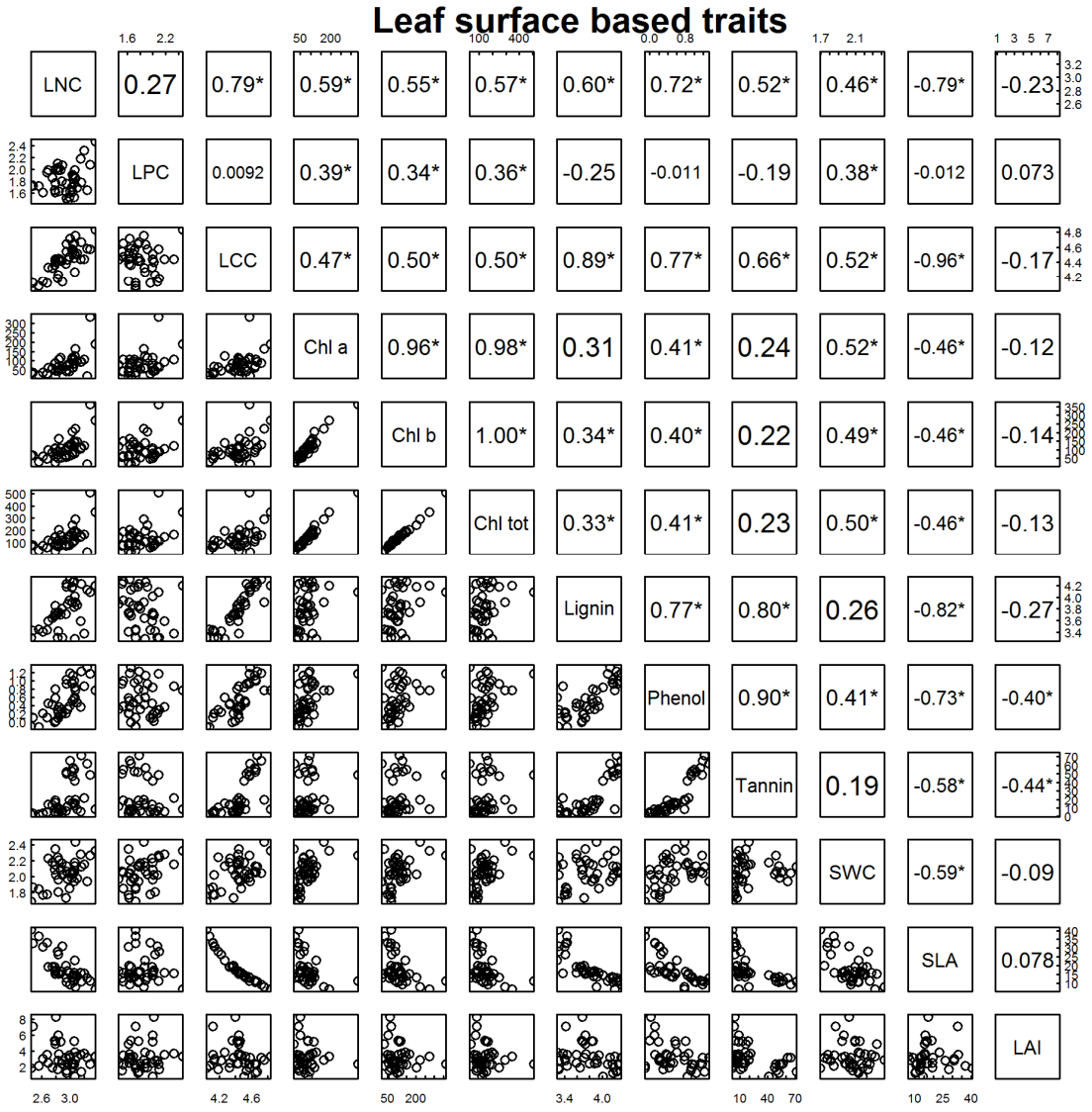
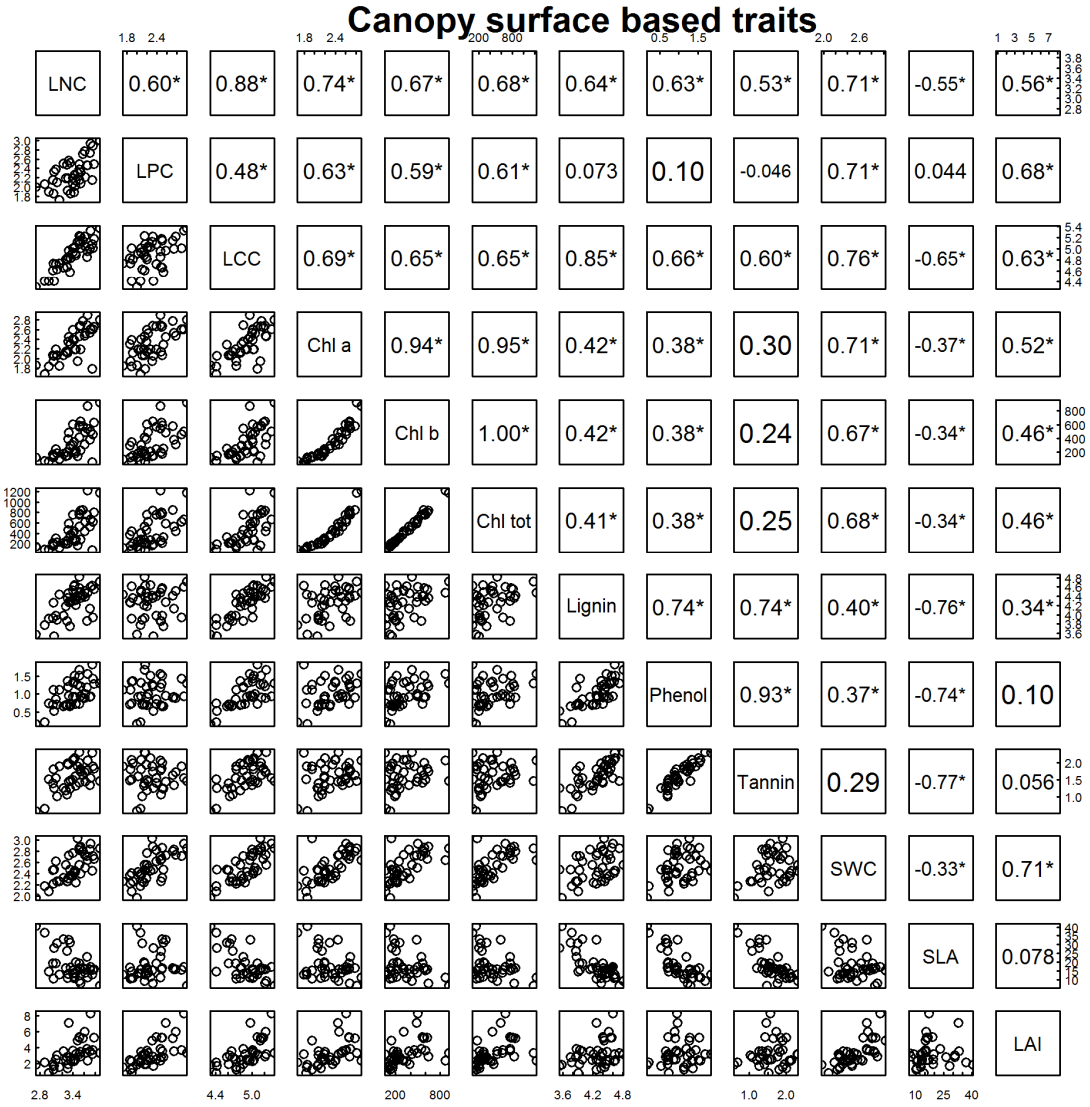


Figure S5. Scatterplots and correlation coefficients for all trait pairs, expressed on canopy surface basis, as well as for SLA and LAI. Correlations significant at $p<0.05$ are indicated with an asterisk.



Supplementary Data 3: Plot Co-Variates Correlation

Figure S6. Distribution of plot co-variate values.

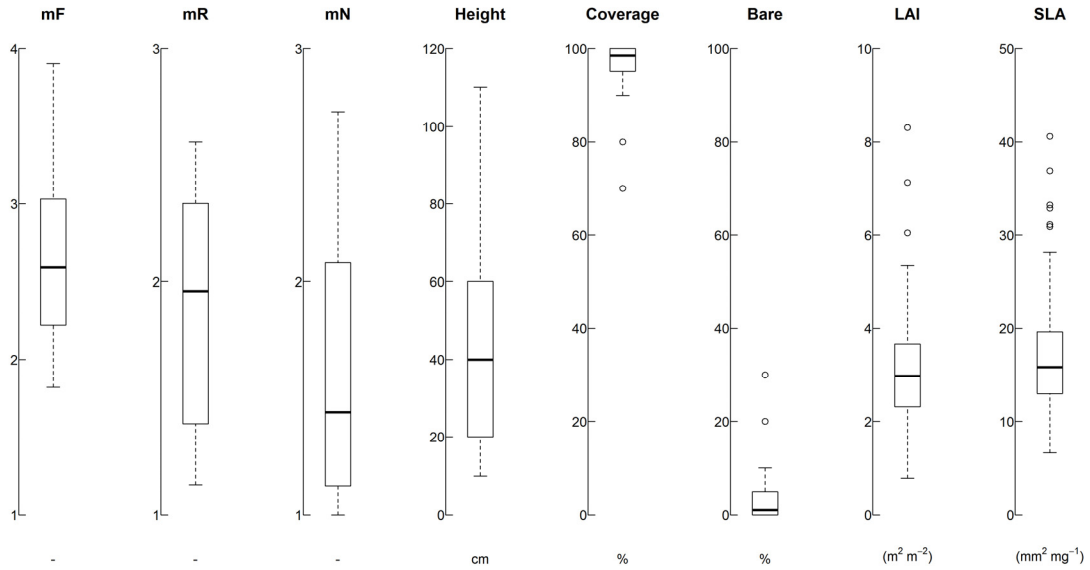
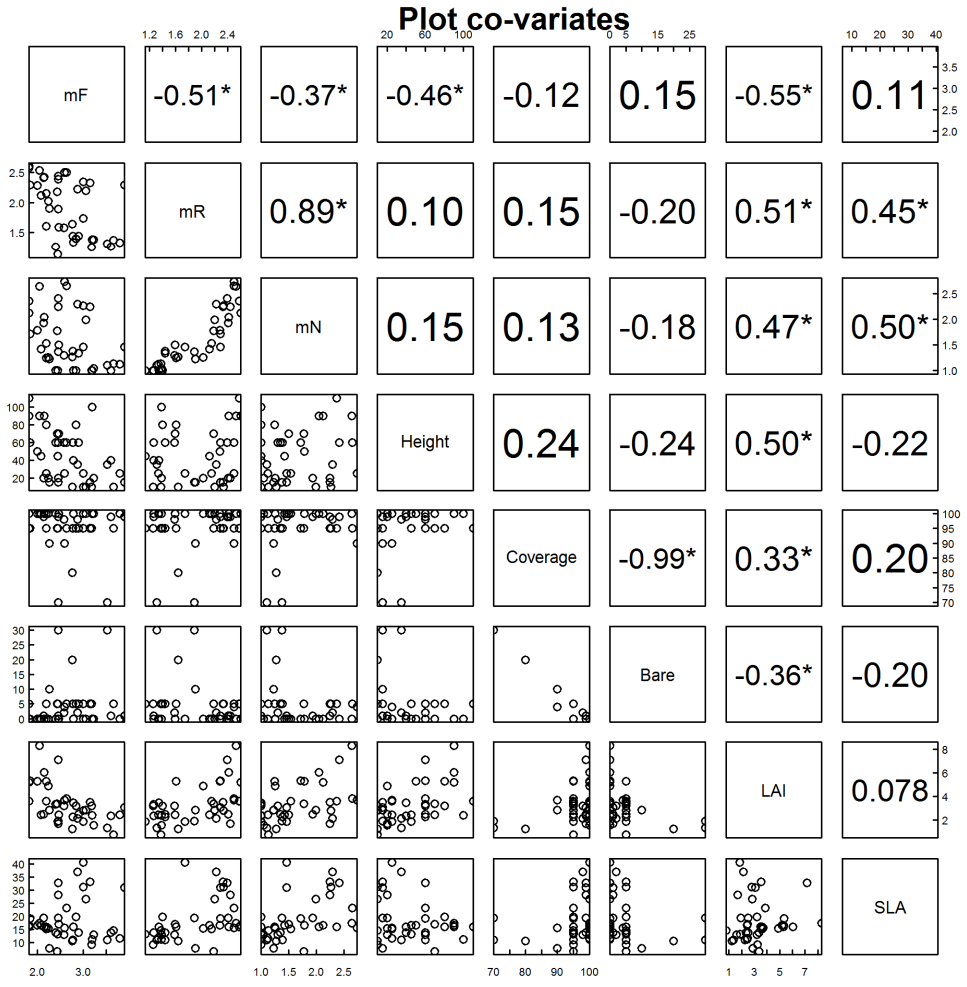
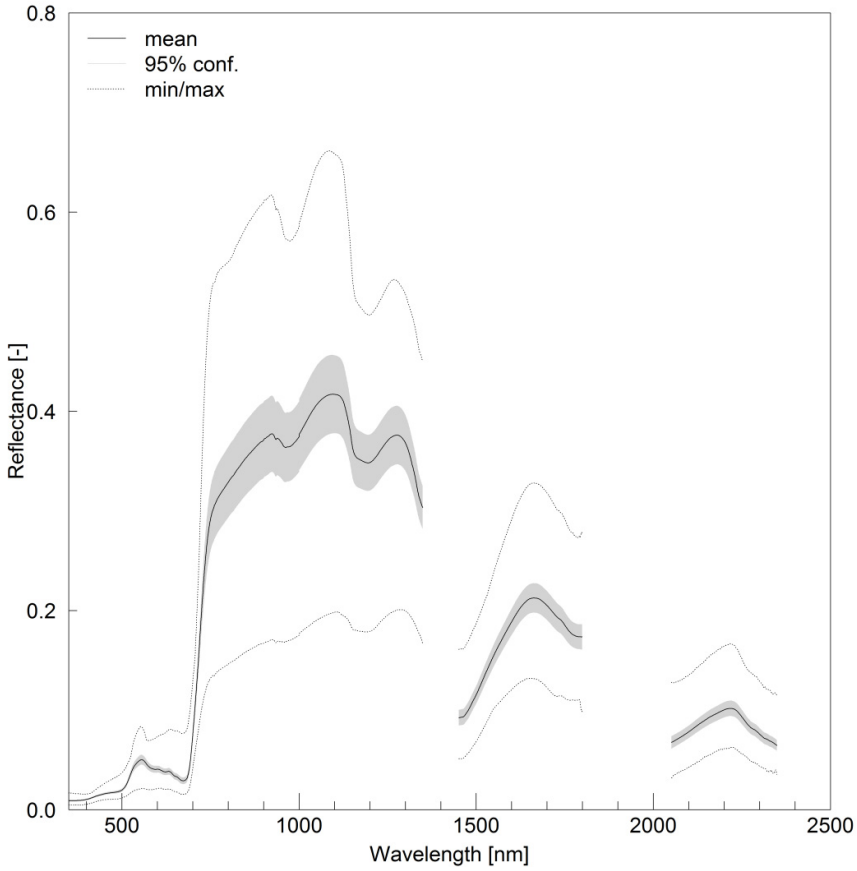


Figure S7. Scatterplots and correlation coefficients for the plot co-variates.



Supplementary Data 4: Plot Canopy Reflectance

Figure S8. Summary statistics for canopy reflectance.



Supplementary Data 5. PLSR Model Regression Coefficients and Correlation Coefficients

Figure S9. Grey bars: regression coefficients of PLSR models for traits expressed on mass basis, standardized to values between −1 and 1. Black line: Pearson correlation coefficient between trait value and each spectral band.

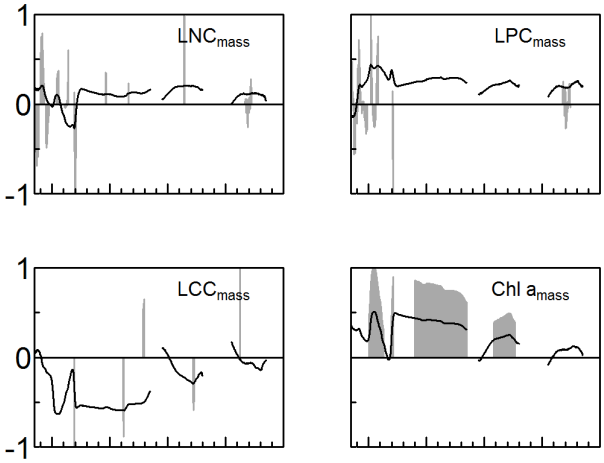


Figure S10. *Cont.*

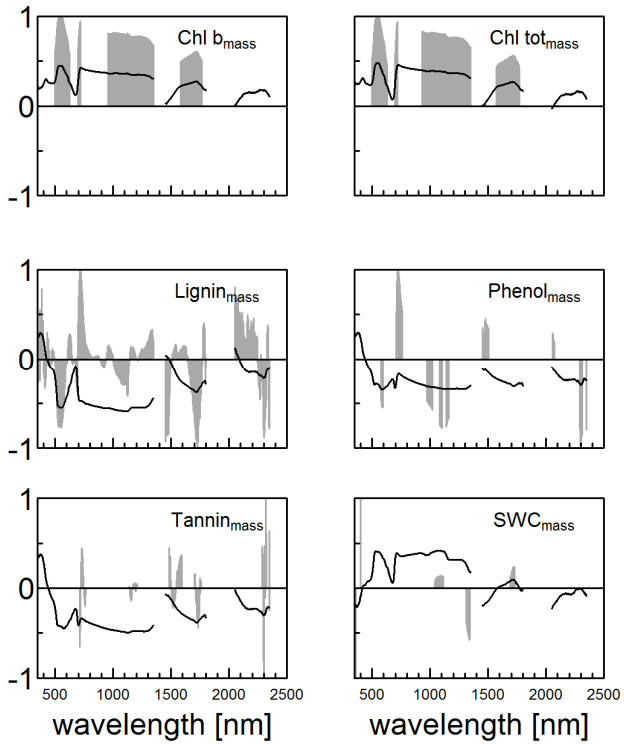


Figure S11. Grey bars: regression coefficients of PLSR models for traits expressed on leaf surface basis, standardized to values between −1 and 1. Black line: Pearson correlation coefficient between trait value and each spectral band.

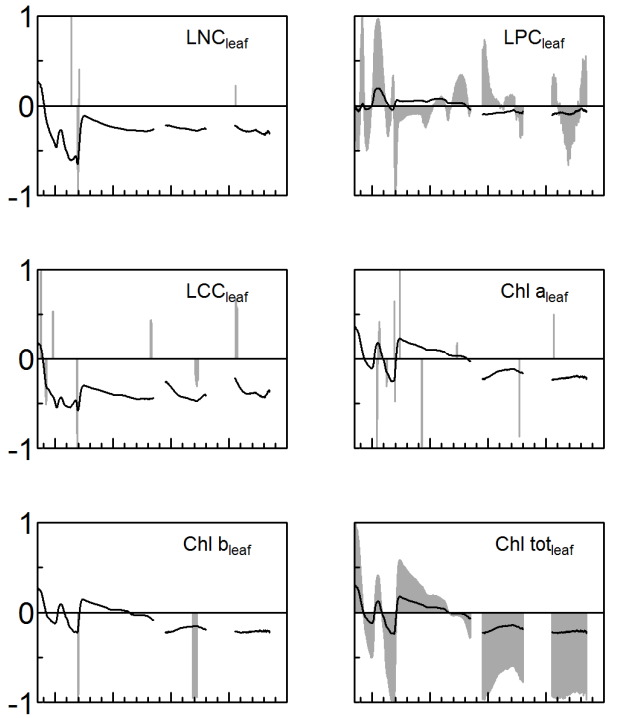


Figure S12. *Cont.*

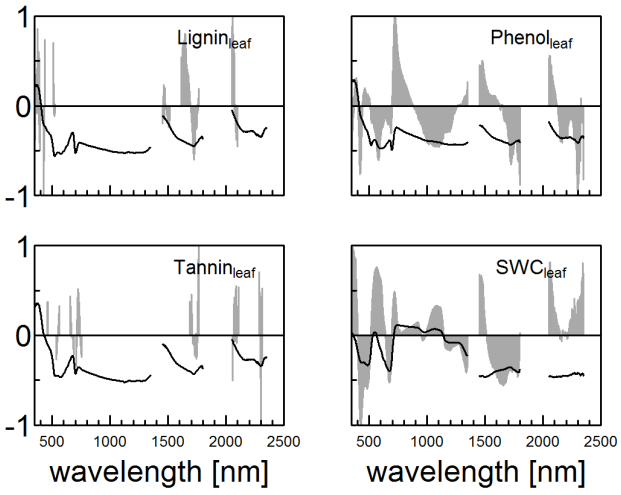


Figure S13. Grey bars: regression coefficients of PLSR models for traits expressed on canopy surface basis, standardized to values between −1 and 1. Black line: Pearson correlation coefficient between trait value and each spectral band.

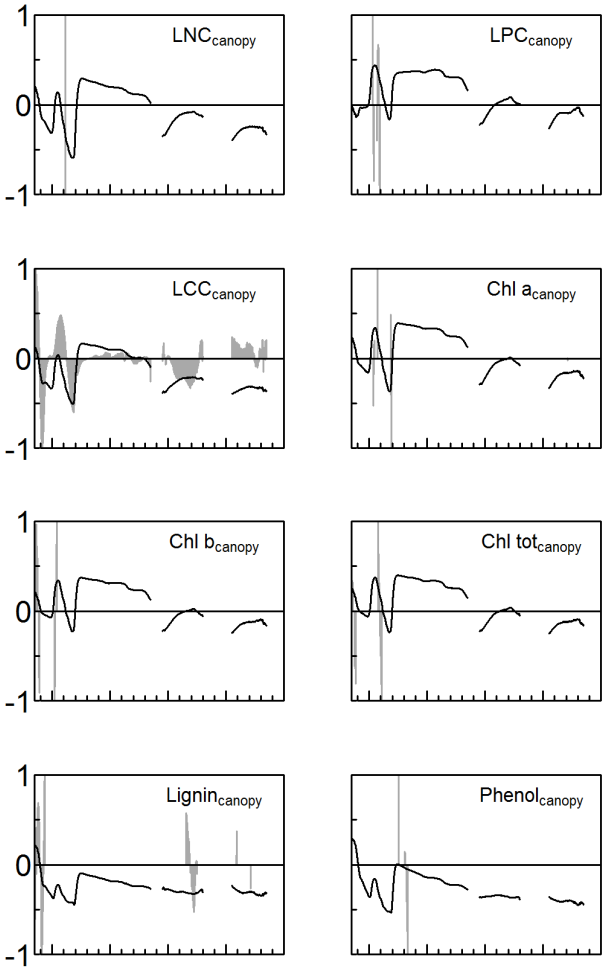
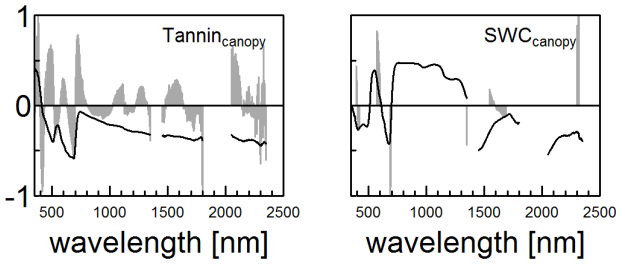


Figure S14. *Cont.*



Supplementary Data 6: Performance Indicators and Summary Statistics PLSR Models

Table S2. PLSR modelling results. Log transformed traits are indicated with an asterisks.

	Mass traits					Leaf Surface Traits					Canopy Surface Traits				
	latent variables	r^2 calibration	r^2 validation	RMSE calibration	RMSE validation	latent variables	r^2 calibration	r^2 validation	RMSE calibration	RMSE validation	latent variables	r^2 calibration	r^2 validation	RMSE calibration	RMSE validation
LNC	8*	0.78	0.56	0.06	0.09	4*	0.71	0.65	0.11	0.12	2*	0.50	0.44	0.19	0.20
LPC	8*	0.81	0.67	0.12	0.16	8*	0.66	0.30	0.13	0.19	4*	0.62	0.51	0.19	0.22
LCC	5*	0.68	0.56	0.01	0.02	7*	0.83	0.74	0.08	0.10	4*	0.45	0.11	0.19	0.24
Chl a	1	0.16	0.05	0.58	0.62	10	0.71	0.39	29.34	42.73	5*	0.51	0.34	0.22	0.25
Chl b	1	0.14	0.04	0.71	0.74	1	0.02	-0.05	64.99	67.52	3	0.33	0.18	177.98	197.55
Total Chl	1	0.15	0.05	0.93	0.98	1	0.09	-0.18	84.17	95.63	3	0.33	0.17	239.37	265.66
Lignin	8	0.84	0.60	19.61	31.14	7*	0.83	0.68	0.13	0.18	6*	0.63	0.36	0.19	0.26
Phenol	4*	0.71	0.60	0.15	0.18	6*	0.78	0.59	0.19	0.26	2*	0.41	0.26	0.28	0.32
Tannin	7	0.89	0.73	0.07	0.12	10	0.94	0.82	5.16	8.94	9*	0.91	0.60	0.12	0.25
SWC	4	0.66	0.56	0.47	0.54	5*	0.50	0.20	0.12	0.15	5*	0.73	0.56	0.13	0.17

Table S3. Coefficient of determination r2 for the PLSR model calibration, for each trait and each of the three trait expressions.

	r^2 Calibration									
	LNC	LPC	LCC	Chl a	Chl b	Total Chl	Lignin	Phenol	Tannin	SWC
mass	0.78	0.81	0.68	0.16	0.14	0.15	0.84	0.71	0.89	0.66
leaf	0.71	0.66	0.83	0.71	0.02	0.09	0.83	0.78	0.94	0.50
canopy	0.50	0.62	0.45	0.51	0.33	0.33	0.63	0.41	0.91	0.73

Table S4. Coefficient of determination r2 for the PLSR model validation, for each trait and each of the three trait expressions.

	r^2 Validation									
	LNC	LPC	LCC	Chl a	Chl b	Total Chl	Lignin	Phenol	Tannin	SWC
mass	0.56	0.67	0.56	0.05	0.04	0.05	0.60	0.60	0.73	0.56
leaf	0.65	0.30	0.74	0.39	-0.05	-0.18	0.68	0.59	0.82	0.20
canopy	0.44	0.51	0.11	0.34	0.18	0.17	0.36	0.26	0.60	0.56

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