

Supplementary material for:

C₄ trees have broader niches than their close C₃ relatives

Sophie N. R. Young, Luke T. Dunning, Hui Liu, Carly J. Stevens, Marjorie R. Lundgren

CONTENTS:

Supplementary Figures

- Fig S1. Map of occurrence data flagged by CoordinateCleaner
- Fig S2. Map of occurrence data by data source
- Fig S3. Map of distributions of Hawaiian herbs, shrubs and trees by photosynthetic type
- Fig S4. Complete phylogeny of all species whose data was obtained in this study
- Fig S5. Principal components 1 and 2 of a Principal Component Analysis (PCA) of environmental variables for all growth forms
- Fig S6. Principal components 3 and 4 of a Principal Component Analysis (PCA) of environmental variables for all growth forms
- Fig S7. Principal Component Analysis (PCA) of soil variables for C₃ and C₄ trees
- Fig S8. Histogram of principal component 3 of a Principal Component Analysis (PCA) of environmental variables for C₃ and C₄ trees.
- Fig S9. Distribution of C₃ and C₄ trees by elevation and environmental variables correlated with elevation.

Supplementary Tables

- Table S1. Issues identified by GBIF which resulted in a record being removed from this analysis
- Table S2. Summary of sequence data used and accession numbers
- Table S3. Environmental variables correlated to PCA axes (trees only)
- Table S4. Environmental variables correlated to PCA axes (all lifeforms)
- Table S5. Soil variables correlated to PCA axes (trees only)

SUPPLEMENTARY FIGURES

Figure S1

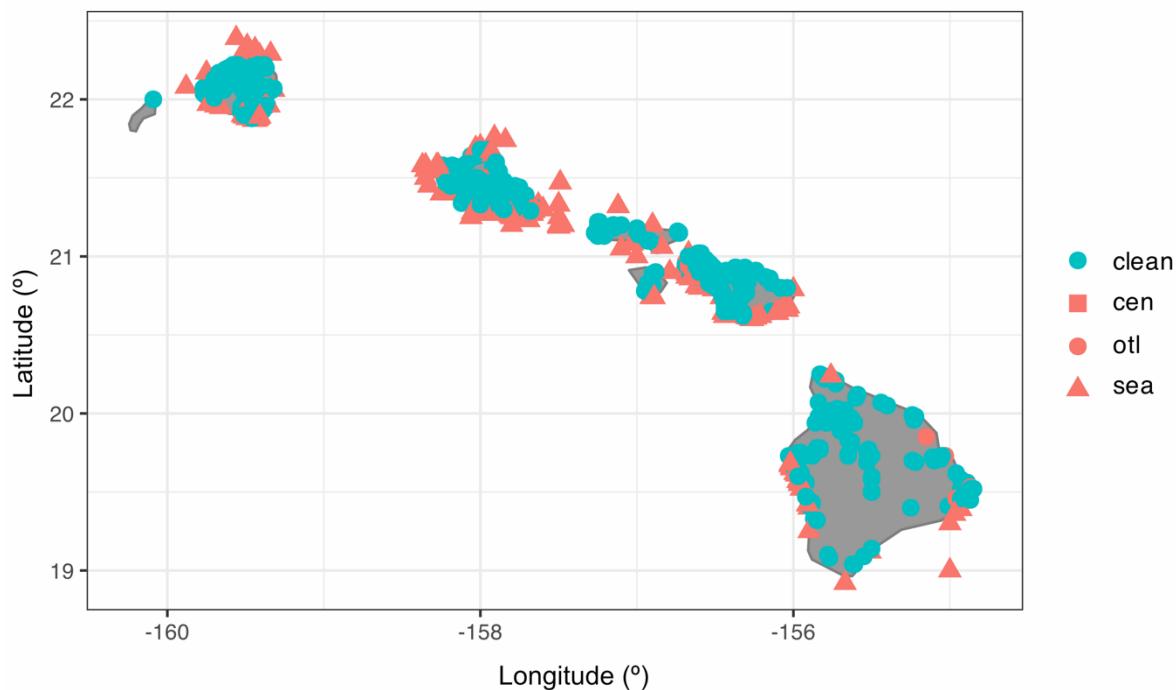


Fig S1. Map of occurrence data flagged by CoordinateCleaner. Occurrence data for species in Euphorbiaceae on the Hawaiian Islands were processed using the CoordinateCleaner package in R. 277 coordinates were flagged (pink) including those located on country centroids (cen, n = 1, squares), species outliers (otl, n = 22, circles), or in the sea (sea, n = 262, triangles), and removed prior to further analysis. Remaining coordinates (n = 1091) are designated as ‘clean’ (blue circles).

Figure S2

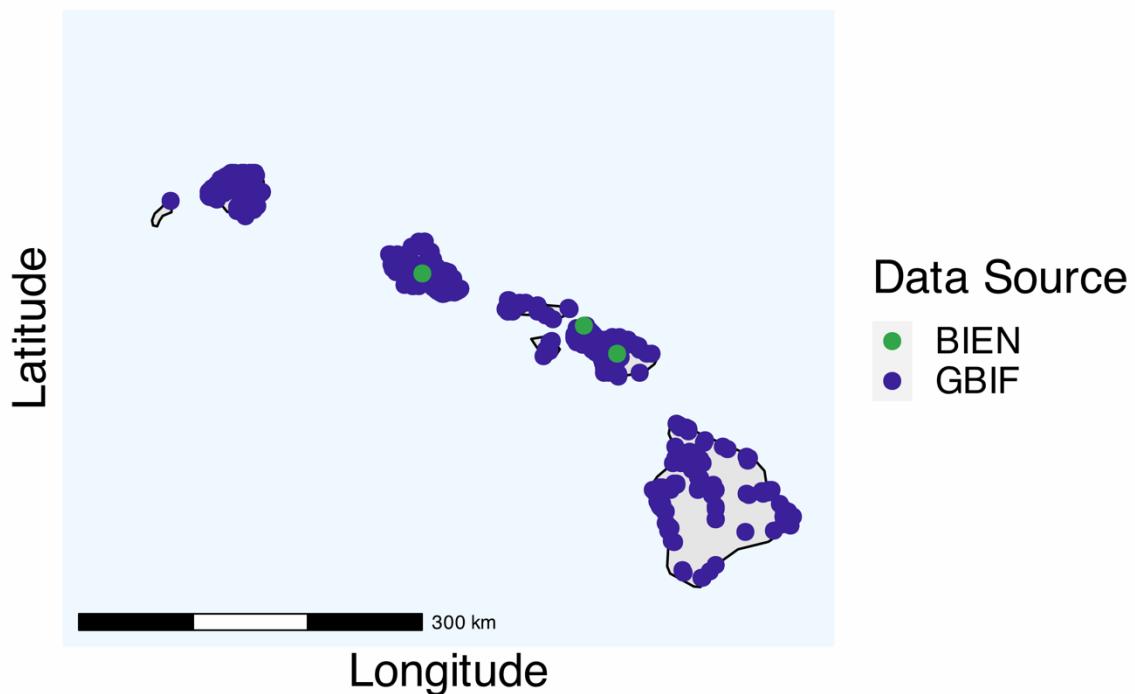


Fig S2. Map of occurrence data by data source. Cleaned occurrence data are shown for species in Euphorbiaceae that were obtained from either the Botanical Information and Ecology Network (BIEN, n = 3, green) or the Global Biodiversity Information Facility (GBIF, n = 687, blue). Duplicate records (*i.e.* those that appeared in both datasets, or twice in one dataset) have been removed.

Figure S3

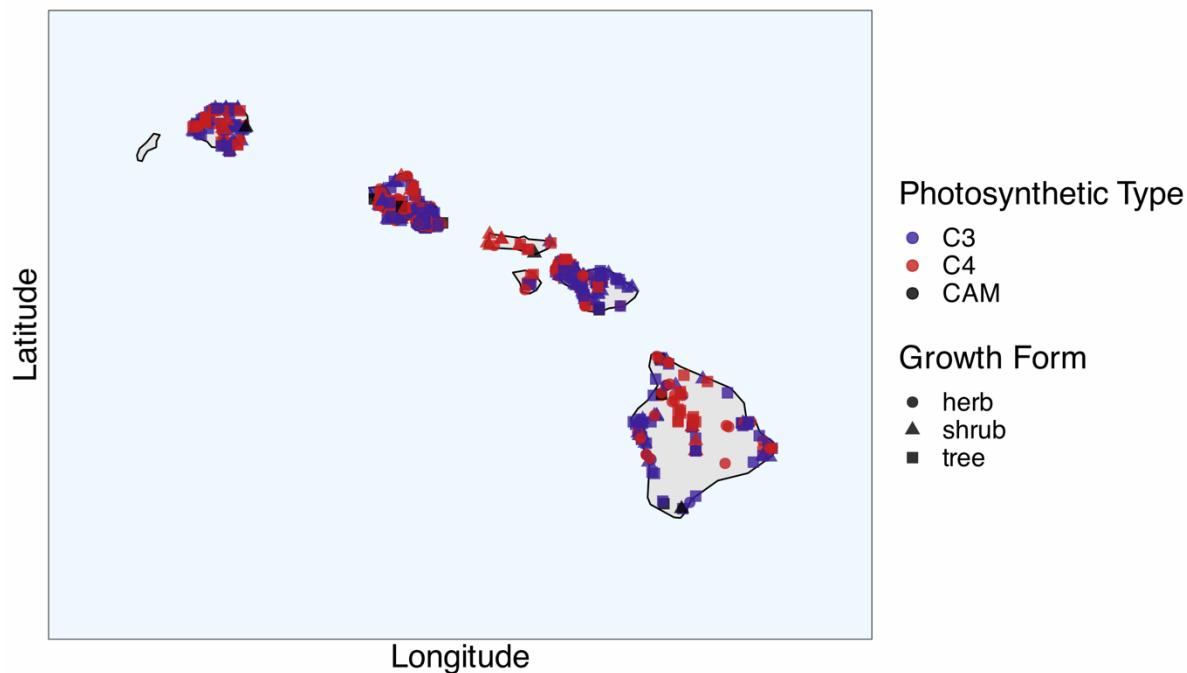


Fig S3. Map of distributions of Hawaiian herbs, shrubs and trees by photosynthetic type.

Occurrence data for herbs ($n = 199$, representing 9 species, circles), shrubs ($n = 170$, representing 18 species, triangles) and trees ($n = 283$, representing 25 species, squares) in Euphorbiaceae are shown on a map of the Hawaiian Islands. Points are coloured according to the photosynthetic type assigned to each species: C₃ (blue), C₄ (red) or CAM (black).

Figure S4

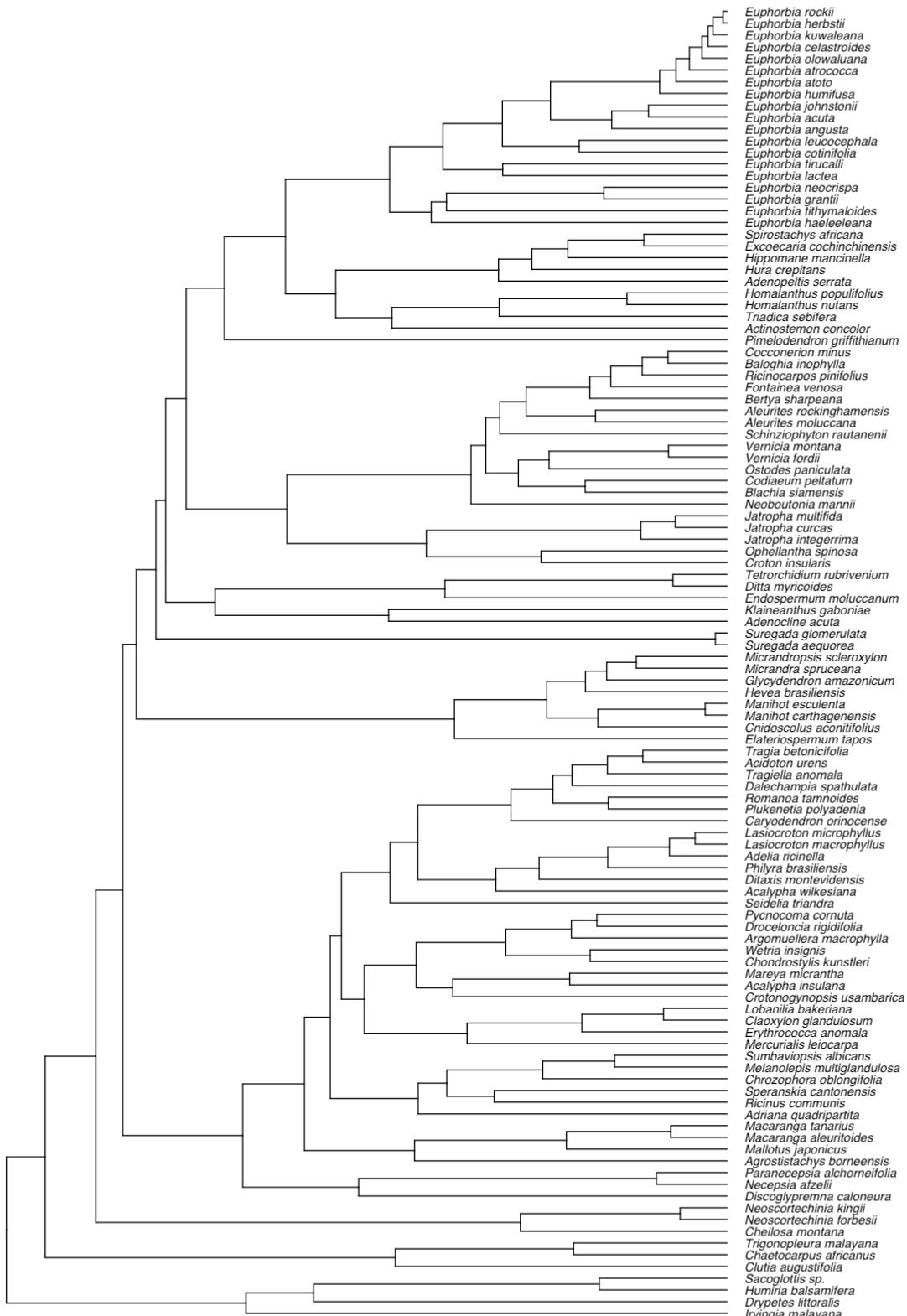


Fig S4. Complete phylogeny of all species whose data was obtained in this study. Phylogenetic tree generated using previously published data for 112 species in Euphorbiaceae.

Figure S5

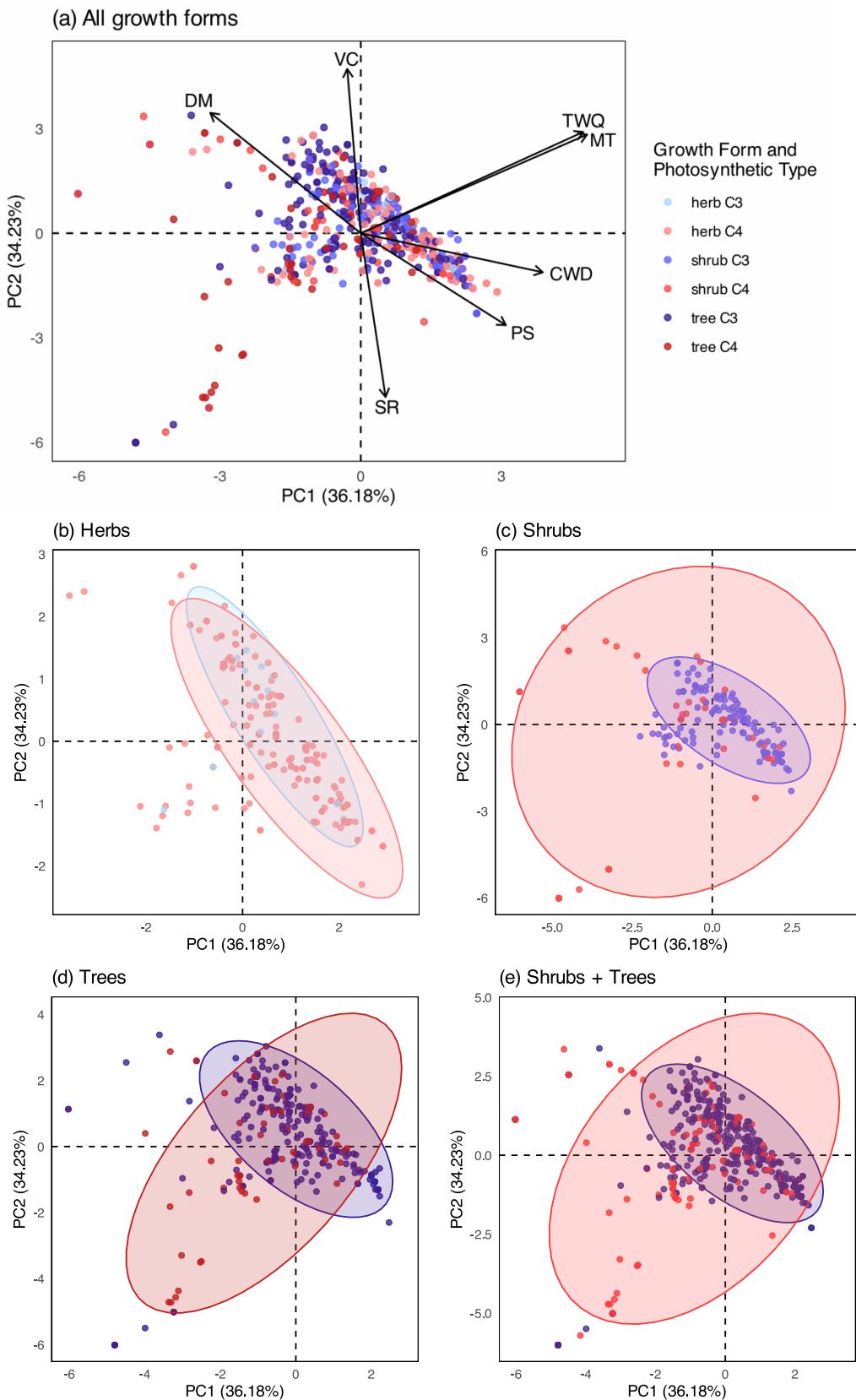


Fig S5. Principal components 1 and 2 of a Principal Component Analysis (PCA) of environmental variables for all growth forms. (a) PCA plot showing C₃ (blue) and C₄ (red) herb, shrub, and tree species in Euphorbiaceae. Arrows show the loading of each of the 7 environmental variables used in the PCA. Abbreviations are as follows: MT=minimum temperature of the coldest month, TWQ= temperature of the wettest quarter, DM=precipitation of the driest month, PS=precipitation seasonality, SR=solar radiation, VC=vegetation cover, CWD=climatic water deficit. Data are also shown on individual plots separated by growth form, with 95% confidence ellipses calculated for each group. (b) C₃ (n = 21) and C₄ (n = 178) herbaceous species, (c) C₃ (n = 119) and C₄ (n = 47) shrub species, C₃ (n = 202) and C₄ (n = 66) tree species, and (e) C₃ (n = 331) and C₄ (n = 113) woody (herb and shrub) species.

Figure S6

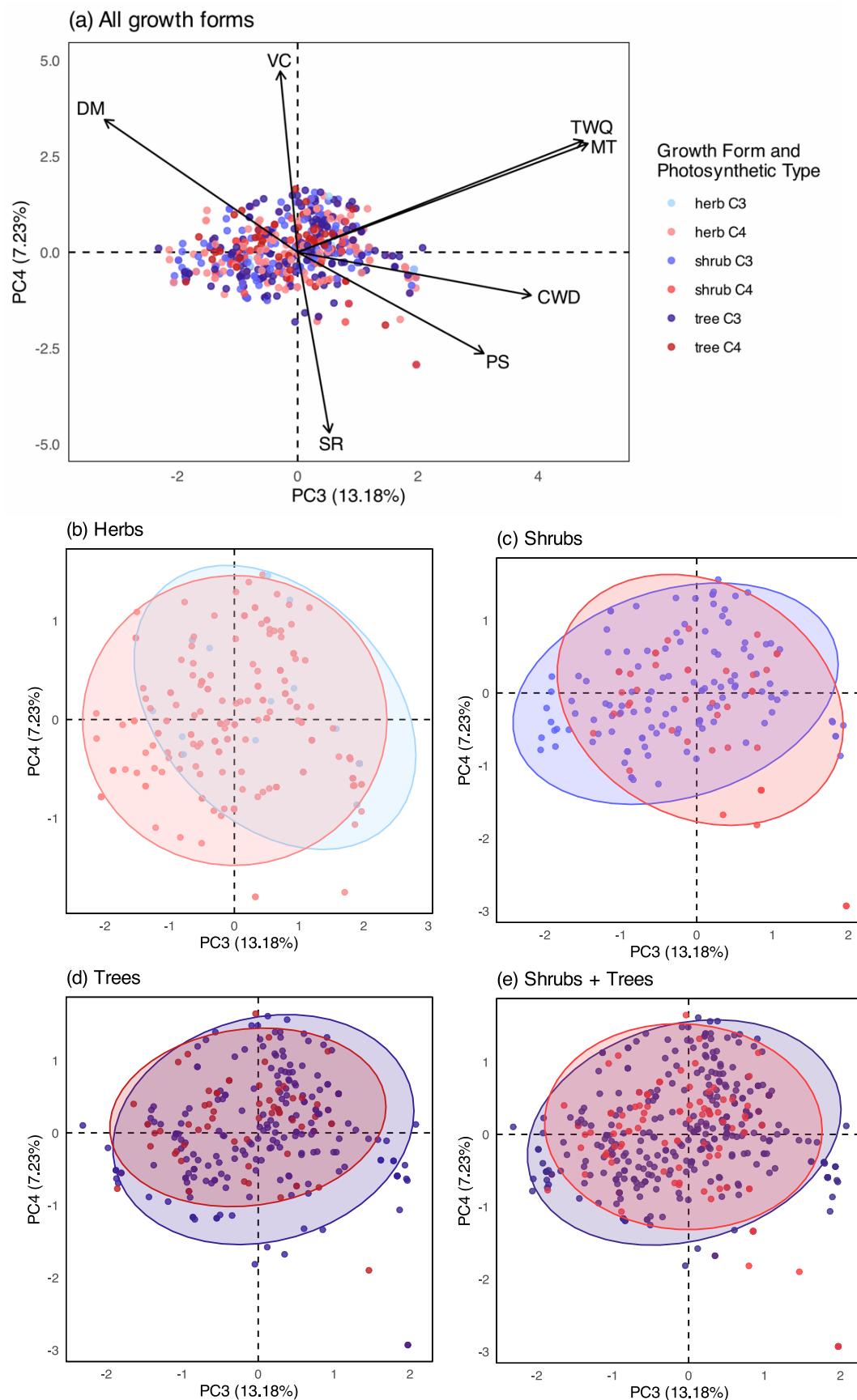


Fig S6. Principal components 3 and 4 of a Principal Component Analysis (PCA) of environmental variables for all growth forms. (a) PCA plot showing C₃ (blue) and C₄ (red) herb, shrub, and tree species in Euphorbiaceae. Arrows show the loading of each of the 7 environmental variables used in the PCA. Abbreviations are as follows: MT=minimum temperature of the coldest month, TWQ= temperature of the wettest quarter, DM=precipitation of the driest month, PS=precipitation seasonality, SR=solar radiation, VC=vegetation cover, CWD=climatic water deficit. Data are also shown on individual plots separated by growth form, with 95% confidence ellipses calculated for each group. (b) C₃ (n = 21) and C₄ (n = 178) herbaceous species, (c) C₃ (n = 119) and C₄ (n = 47) shrub species, C₃ (n = 202) and C₄ (n = 66) tree species, and (e) C₃ (n = 331) and C₄ (n = 113) woody (herb and shrub) species.

Figure S7

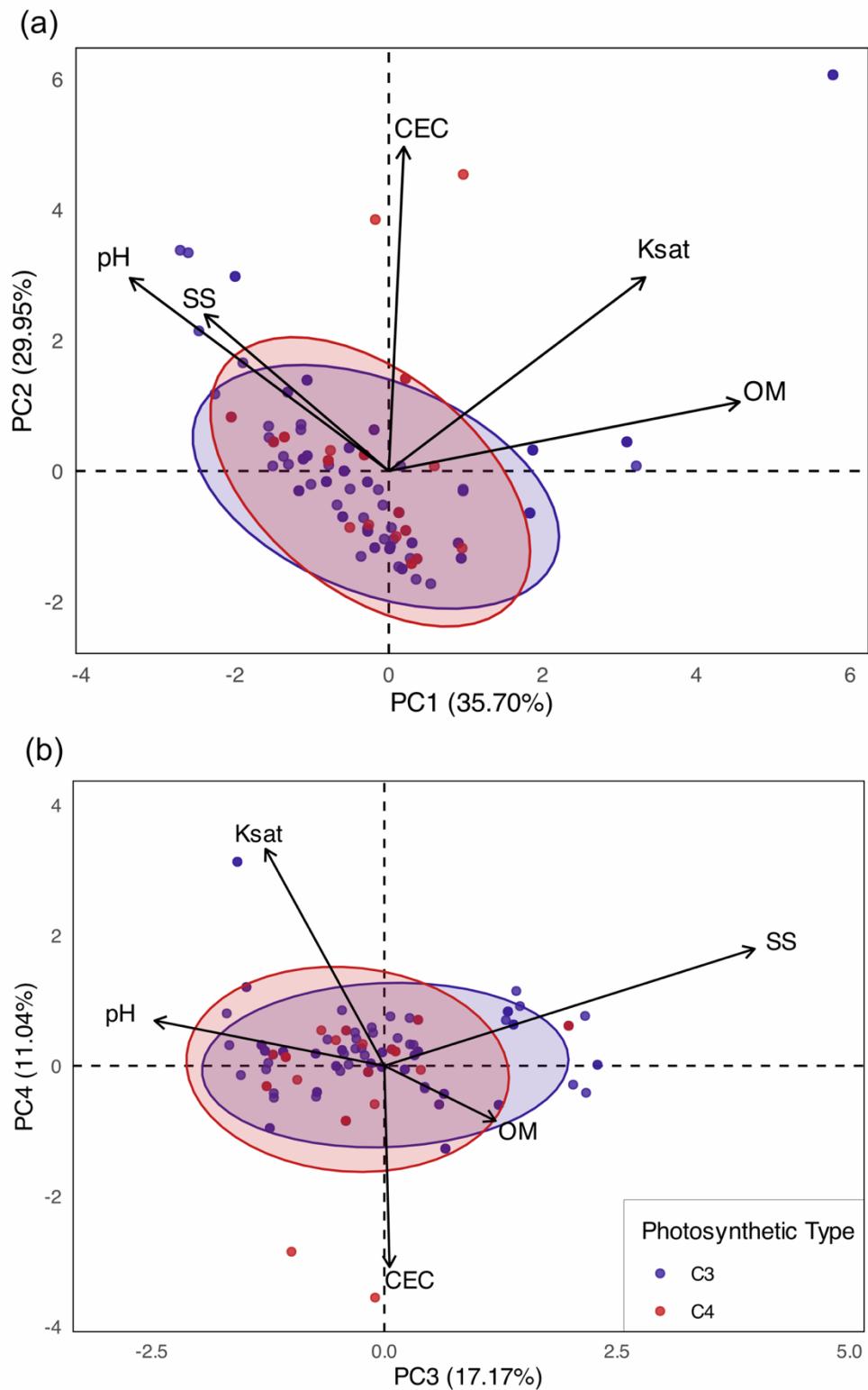


Fig S7. Principal component analysis (PCA) of soil variables for C₃ and C₄ trees. (a) Principal Components (PCs) 1 and 2, and, (b) PCs 3 and 4 of a PCA conducted on 5 soil variables for C₃ (blue) and C₄ (red) trees. Arrows show the loading of each of the 5 variables. Abbreviations are as follows: CEC = cation exchange capacity, Ksat = water permeability, SS = shrink-swell potential (soil stability), OM = organic matter. 95% confidence ellipses were calculated for each group.

Figure S8

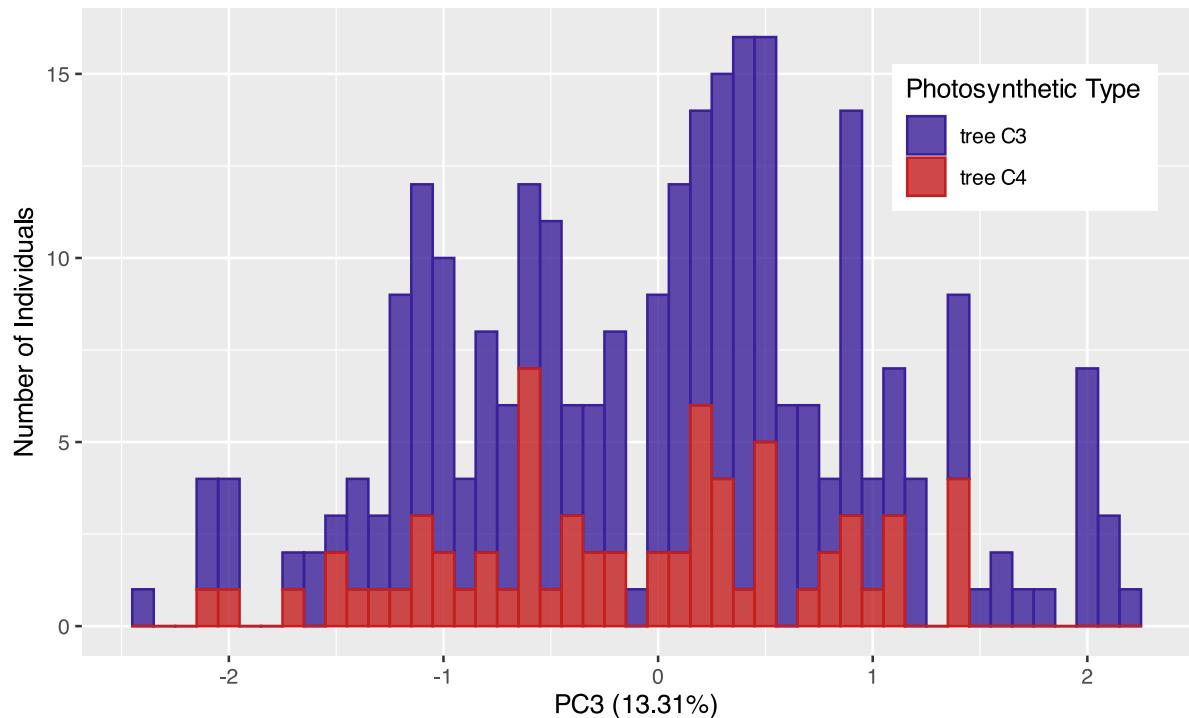


Fig S8. Histogram of Principal Component 3 of a Principal Component Analysis (PCA) of environmental variables for C₃ and C₄ trees. Histogram showing C₃ (blue, n = 202) and C₄ (red, n = 66) tree species in Euphorbiaceae.

Figure S9

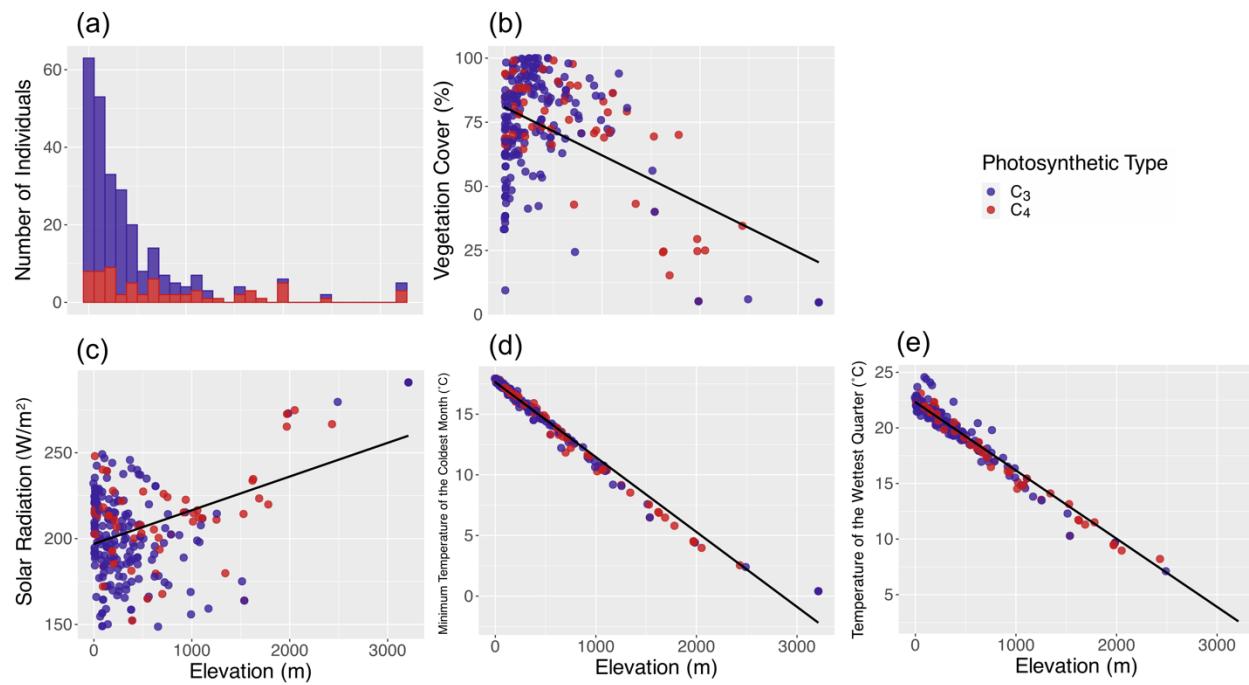


Fig S9. Distribution of C_3 and C_4 trees by elevation, and environmental variables correlated with elevation. (a) Histogram of the distribution of C_3 (blue) and C_4 (red) trees by elevation. Scatterplots of the relationship between elevation and 4 environmental variables are shown: (b) vegetation cover, (c) solar radiation, (d) minimum temperature of the coldest month and (e) temperature of the wettest quarter. Linear regression lines (black) were fitted to the data in plots b-e.

SUPPLEMENTARY TABLES

Table S1. Issues identified by GBIF which resulted in a record being removed from this analysis

code	issue	description	type
conti	CONTINENT_INVALID	Uninterpretable continent values found.	occurrence
cucdmis	COUNTRY_COORDINATE_MISMATCH	The interpreted occurrence coordinates fall outside of the indicated country.	occurrence
cuviv	COUNTRY_INVALID	Uninterpretable country values found.	occurrence
cum	COUNTRY_MISMATCH	Interpreted country for dwc:country and dwc:countryCode contradict each other.	occurrence
preneglat	PRESUMED_NEGATED_LATITUDE	Latitude appears to be negated, e.g. 32.3 instead of -32.3	occurrence
preneglon	PRESUMED_NEGATED_LONGITUDE	Longitude appears to be negated, e.g. 32.3 instead of -32.3	occurrence
preswcd	PRESUMED_SWAPPED_COORDINATE	Latitude and longitude appear to be swapped.	occurrence
txmatfuz	TAXON_MATCH_FUZZY	Matching to the taxonomic backbone can only be done using a fuzzy, non-exact match.	occurrence
cdpi	COORDINATE_PRECISION_INVALID	Indicates an invalid or very unlikely coordinatePrecision	occurrence

Table S2. summary of sequence data used and accession numbers

Species	rbcL		matK		trnL		Total (bp)
	Length (bp)	Accession	Length (bp)	Accession	Length (bp)	Accession	
Acalypha_insulana	1331	AB233854	1203	AB233750	0	-	2534
Acalypha_wilkesiana	599	KU243027	0	-	0	-	599
Acidoton urens	1321	AB267913	1194	AB268017	0	-	2515
Actinostemon concolor	1324	AB233883	1203	AB233779	0	-	2527
Adelia_ricinella	1315	AB267914	1203	AB268018	1016	AY79473 7	3534
Adenocline_acuta	1325	AB233874	1197	AB233770	0	-	2522
Adenopeltis_serrata	1324	AB267954	1203	AB268058	791	AY79463 3	3318
Adriana_quadripartita	1325	AB233855	1206	AB233751	0	-	2531
Agrostistachys_borneensis	1331	AB233856	1203	AB233752	0	-	2534
Aleurites_moluccana	1321	AY794883	1218	LK021377	904	AY79470 9	3443
Aleurites_rockinghamensis	0	-	0	-	743	KC42843 7	743
Argomuellera_macrophylla	1325	AB267915	1203	AB268019	986	AY79476 9	3514
Baloghia_inophylla	1331	AB233875	1203	AB233771	899	AY79470 7	3433
Bertya_sharpeana	1324	AB267935	1215	AB268039	0	-	2539
Blachia_siamensis	1324	AB267936	1203	AB268040	873	AY79472 7	3400
Caryodendron_orinocense	1325	AB233857	1203	AB233753	997	AY79476 0	3525
Chaetocarpus_africanus	1325	AB233858	1191	AB233754	854	AY79480 9	3370
Cheilosia_montana	1331	AB267910	1203	AB268014	0	-	2534
Chondrostylis_kunstleri	1331	AB233859	1203	AB233755	0	-	2534
Chrozophora Oblongifolia	1325	AB233860	1203	AB233756	0	-	2528
Claoxylon_glandulosum	0	-	1209	LK021406	998	HG97184 3	2207
Clutia_augustifolia	1321	AB233861	1191	AB233757	0	-	2512
Cnidoscolus_aconitifolius	1325	AB267937	1203	AB268041	946	MH93528 3	3474
Coccoherion_minus	1323	AB267938	1203	AB268042	0	-	2526
Codiaeum_peltatum	1331	AB233876	1203	AB233772	0	-	2534
Croton_insularis	1331	AB233877	1203	AB233773	697	AY97130 8	3231
Crotonogynopsis_usambarica	1331	AB233862	1209	AB233758	1008	HG97185 3	3548
Dalechampia_spathulata	1331	AB233863	1188	AB233759	1080	Y794754	3599
Discoglypremma_caloneura	1324	AB267916	1209	AB268020	1095	AY79480 2	3628
Ditaxis_montevidensis	1324	AB233865	1203	AB233761	0	-	2527
Ditta_myricoides	1311	AB267939	1212	AB268043	1006	AY79467 5	3529
Droceloncia_rigidifolia	1318	AB267917	1203	AB268021	0	-	2521
Drypetes_littoralis	1331	AB233926	1203	AB233822	0	-	2534
Elateriospermum_tapos	1322	AB267940	1203	AB268044	964	AY79467 8	3489
Endospermum_moluccanum	1331	AB267941	1206	AB268045	976	AY79467 1	3513
Erythrococca_anomala	1324	AB267918	1215	AB268022	0	-	2539
Euphorbia_acuta	1300	JN249261	1203	HQ645674	982	JN249596	3485
Euphorbia_angusta	0	-	1203	HQ645678	381	HQ64552 8	1584
Euphorbia_atoto	1331	AB267955	1203	AB268059	402	HQ64553 6	2936

Species	rbcL		matK		trnL		Total (bp)
	Length (bp)	Accession	Length (bp)	Accession	Length (bp)	Accession	
<i>Euphorbia_atrococca</i>	0	-	0	-	1031	MH49036 5	1031
<i>Euphorbia_celastroides</i>	1106	JN249280	1203	HQ645698	1053	JN249615	3362
<i>Euphorbia_grantii</i>	1331	AB233888	1200	AB233784	1032	JN249654	3563
<i>Euphorbia_haeleeleana</i>	0	-	1221	KC019490	0	-	1221
<i>Euphorbia_herbstii</i>	0	-	0	-	1036	MH49044 6	1036
<i>Euphorbia_humifusa</i>	1331	AB233884	1203	AB233780	406	HQ64558 7	2940
<i>Euphorbia_johnstonii</i>	0	-	1203	HQ645742	386	HQ64559 2	1589
<i>Euphorbia_kuwaleana</i>	0	-	1203	HQ645743	1048	MH49036 1	2251
<i>Euphorbia_leucocephala</i>	1300	JN249340	637	GU214862	978	JN249672	2915
<i>Euphorbia_neocrispa</i>	1324	AB267958	1224	AB268062	0	-	2548
<i>Euphorbia_olowaluana</i>	0	-	1203	HQ645766	1042	MH49036 3	2245
<i>Euphorbia_rockii</i>	0	-	1004	HQ645791	1022	MH49048 9	2026
<i>Euphorbia_tithymaloides</i>	1331	AB267959	1203	AB268063	0	-	2534
<i>Excoecaria_cochinchinensis</i>	1331	AB233885	1203	AB233781	959	AY79461 9	3493
<i>Fontainea_venosa</i>	1324	AB233878	1203	AB233774	872	AY79470 8	3399
<i>Glycydendron_amazonicum</i>	1324	AB267942	1209	AB268046	964	AY79468 1	3497
<i>Hevea_brasiliensis</i>	1316	NC_15308	1197	NC_15308	988	NC_1530 8	3501
<i>Hippomane_mancinella</i>	1324	AB267956	1203	AB268060	983	AY79461 6	3510
<i>Homalanthus_nutans</i>	1331	AB267957	1203	AB268061	961	JN249590	3495
<i>Homalanthus_populifolius</i>	572	KM392269	757	KM392242	0	-	1329
<i>Humiria_balsamifera</i>	1324	AB233889	1197	AB233785	0	-	2521
<i>Hura_crepitans</i>	1331	AB233886	1203	AB233782	984	AY79463 6	3518
<i>Irvingia_malayana</i>	1331	AB233892	1195	AB233788	0	-	2526
<i>Jatropha_integerrima</i>	1331	AB233879	1215	AB233775	1034	AY79468 5	3580
<i>Jatropha_multifida</i>	687	KP898357	749	LC461834	937	KP868720	2373
<i>Jatropha_curcas</i>	1315	NC_12224	1212	NC_12224	1024	NC_1222 4	3551
<i>Klaineanthus_gaboniae</i>	1331	AB267944	1203	AB268048	887	AY79466 8	3421
<i>Lasiocroton_macrophyllus</i>	1324	AB267919	1203	AB268023	0	-	2527
<i>Lasiocroton_microphyllus</i>	1320	AB267920	1203	AB268024	990	HG97189 3	3513
<i>Lobanilia_bakeriana</i>	1324	AB267921	1209	AB268025	1022	AY79477 9	3555
<i>Macaranga_aleuritoides</i>	1331	AB267922	1203	AB268026	988	Q899191	3522
<i>Macaranga_tanarius</i>	1331	MW297079	1203	MW297079	994	MW29707 9	3528
<i>Mallotus_japonicus</i>	1309	MW244068	1203	MW244068	990	MW24406 8	3502
<i>Manihot_carthagensis</i>	1331	MK430314. 1	0	-	964	EU518901	2295
<i>Manihot_esculenta</i>	1313	NC_10433	1209	NC_10433	1000	NC_1043 3	3522
<i>Mareya_micrantha</i>	1324	AB267924	1197	AB268028	1026	AY79477 4	3547
<i>Melanolepis_multiglandulosa</i>	1331	AB267925	1203	AB268029	988	AY79473 3	3522
<i>Mercurialis_leiocarpa</i>	1331	AB233867	1203	AB233763	0	-	2534
<i>Micrandra_spruceana</i>	1323	AB267945	1212	AB268049	0	-	2535

Species	rbcL		matK		trnL		Total (bp)
	Length (bp)	Accession	Length (bp)	Accession	Length (bp)	Accession	
<i>Micrandropsis_scleroxylon</i>	1321	AB267946	1206	AB268050	0	-	2527
<i>Necepsia_afzelii</i>	1324	AB233868	1203	AB233764	986	DQ99151 4	3513
<i>Neoboutonia_mannii</i>	1324	AB233881	1215	AB233777	889	AY79472 3	3428
<i>Neoscoretechinia_forbesii</i>	1322	AB267911	1197	AB268015	0	-	2519
<i>Neoscoretechinia_kingii</i>	1322	AB267912	1203	AB268016	971	AY79480 6	3496
<i>Ophellantha_spinosa</i>	1324	AB267947	1209	AB268051	685	AY97134 4	3218
<i>Ostodes_paniculata</i>	1322	AB267948	1215	AB268052	881	AY79472 5	3418
<i>Paranecepsia_alchorneifolia</i>	1315	AB267926	1203	AB268030	0	-	2518
<i>Philyra_brasiliensis</i>	1324	AB267927	1209	AB268031	1001	AY79474 0	3534
<i>Pimelodendron_griffithianum</i>	1331	AB233887	1187	AB233783	0	-	2518
<i>Plukenetia_polyadenia</i>	1324	AB267928	1194	AB268032	0	-	2518
<i>Pycnocoma_cornuta</i>	1324	AB233870	1203	AB233766	0	-	2527
<i>Ricinocarpus_pinifolius</i>	1324	AB267949	1212	AB268053	0	-	2536
<i>Ricinus_communis</i>	1331	NC_16736	1203	NC_16736	981	NC_1673 6	3515
<i>Romanoa_tamnoides</i>	1320	AB233872	1194	AB233768	1037	Y794757	3551
<i>Sacoglottis_sp.</i>	1323	AB233890	1197	AB233786	0	-	2520
<i>Schinziophyton_rautanenii</i>	1324	AB233882	1215	AB233778	0	-	2539
<i>Seidelia_triantha</i>	1324	AB267929	1203	AB268033	983	AY79476 2	3510
<i>Speranskia_cantonensis</i>	1326	AB267930	1203	AB268034	1025	AY79473 5	3554
<i>Spirostachys_africana</i>	1324	AB267960	1203	AB268064	977	Y794621	3504
<i>Sumbaviopsis_albicans</i>	1324	AB267931	1209	AB268035	986	Y794732	3519
<i>Suregada_aequorea</i>	1331	AB267950	1203	AB268054	0	-	2534
<i>Suregada_glomerulata</i>	1331	AB267951	1203	AB268055	0	-	2534
<i>Tetrorchidium_rubrivenium</i>	1315	AB267952	1212	AB268056	0	-	2527
<i>Tragia_betonicifolia</i>	1324	AB267932	1194	AB268036	0	-	2518
<i>Tragiella_anomala</i>	1324	AB267933	1194	AB268037	1032	AY79475 3	3550
<i>Triadica_sebifera</i>	1331	AB267961	1209	AB268065	1021	Y794649	3561
<i>Trigonopleura_malayana</i>	1331	AB233873	1194	AB233769	0	-	2525
<i>Vernicia_montana</i>	1316	MW297080 .1	1215	MW297080 .1	1049	MW29708 0	3580
<i>Vernicia_fordii</i>	1301	KY628420	0	-	0	-	1301
<i>Wetria_insignis</i>	1324	AB267934	1203	AB268038	0	-	2527

Table S3. Environmental variables correlated to PCA axes (trees only)

	Correlation Coefficient	p-value
Dim 1		
Temperature of the Wettest Quarter	0.8690961	2.699026e-83
Minimum Temperature of the Coldest Month	0.8645576	1.826855e-81
Vegetation Cover	0.7552094	1.035758e-50
Precipitation of the Driest Month	0.3539213	2.508441e-09
Climatic Water Deficit	0.2165732	3.551292e-04
Precipitation Seasonality	-0.2360332	9.570400e-05
Solar Radiation	-0.7002899	7.997929e-41
Dim 2		
Precipitation Seasonality	0.7008964	6.400334e-41
Climatic Water Deficit	0.6665530	8.467696e-36
Minimum Temperature of the Coldest Month	0.4632755	1.155631e-15
Temperature of the Wettest Quarter	0.4485016	1.144784e-14
Solar Radiation	0.4037758	6.238385e-12
Vegetation Cover	-0.2841251	2.274431e-06
Precipitation of the Driest Month	-0.7682722	1.856038e-53
Dim 3		
Climatic Water Deficit	0.6439586	8.730307e-33
Precipitation of the Driest Month	0.2994582	5.898452e-07
Solar Radiation	0.2484549	3.904704e-05
Vegetation Cover	-0.2703221	7.171872e-06
Precipitation Seasonality	-0.5406903	9.516836e-22

Table S4. Environmental variables correlated to PCA axes (all lifeforms)

	Correlation Coefficient	p-value
Dim 1		
Minimum Temperature of the Coldest Month	0.85293675	2.680179e-180
Temperature of the Wettest Quarter	0.83826172	2.393567e-168
Climatic Water Deficit	0.68588944	3.621171e-89
Precipitation Seasonality	0.54686588	1.186145e-50
Solar Radiation	0.09338178	1.877688e-02
Precipitation of the Driest Month	-0.56734656	3.336257e-55
Dim 2		
Vegetation Cover	0.8112428	3.399612e-149
Precipitation of the Driest Month	0.5943777	1.014873e-61
Temperature of the Wettest Quarter	0.5000729	2.339880e-41
Minimum Temperature of the Coldest Month	0.4872993	4.629512e-39
Climatic Water Deficit	-0.1930343	9.924023e-07
Precipitation Seasonality	-0.4538317	1.745728e-33
Solar Radiation	-0.8088022	1.283092e-147
Dim 3		
Climatic Water Deficit	0.6144706	5.582018e-67
Precipitation of the Driest Month	0.3464571	2.731684e-19
Solar Radiation	0.1234679	1.857199e-03
Vegetation Cover	-0.3262061	3.687601e-17
Precipitation Seasonality	-0.5495276	3.165038e-51
Dim 4		
Solar Radiation	0.51276391	9.761776e-44
Vegetation Cover	0.35460097	3.425228e-20
Climatic Water Deficit	-0.09449286	1.740606e-02
Precipitation of the Driest Month	-0.14115937	3.675769e-04
Precipitation Seasonality	-0.28336754	3.738228e-13

Table S5. Soil variables correlated to PCA axes (trees only)

	Correlation Coefficient	p-value
Dim 1		
Organic Matter	0.8703556	2.11E-51
Water Permeability	0.6360706	7.30E-20
Shrink-Swell Potential	-0.4565908	9.02E-10
pH	-0.6425377	2.35E-20
Dim 2		
Cation Exchange Capacity	0.8675693	1.04E-50
Water Permeability	0.5182415	1.39E-12
pH	0.5162413	1.75E-12
Shrink-Swell Potential	0.418833	2.63E-08
Organic Matter	0.1848592	1.82E-02
Dim 3		
Shrink-Swell Potential	0.7374708	3.06E-29
Organic Matter	0.2214541	4.50E-03
Water Permeability	-0.2365187	2.37E-03
pH	-0.4576132	8.18E-10
Dim 4		
Water Permeability	0.494389	1.98E-11
Shrink-Swell Potential	0.2666742	5.80E-04
Cation Exchange Capacity	-0.4585921	7.45E-10