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Throughfall exclusion and fertilization effects on tropical dry forest tree plantations, a large-scale experiment

German Vargas Gutiérrez et al.

Correspondence to: German Vargas Gutiérrez (gevargu@gmail.com)

Supplementary Methods

Methods S1. General additive model for quantifying LAI variation through time.

We examined the total and temporal variation in LAI using several complementary indices: leaf area duration, seasonal LAI enlargement, and maximum LAI. These indices required characterization of the temporal variation in LAI, which describes the variation in the seasonal flushing and shedding of leaves. To do so, we fitted a generalized additive mixed model (GAMM). For each plot, we calculated the mean LAI for a given time-point. We then fitted a GAMM as follows:

$$LAI_{ij} = \beta_0 + \alpha_{0i} + f\left(Day_{ij}\right) + \varepsilon_{ij}$$

$$\alpha_i \sim N(0, SL^2)$$

In this model, each LAI observation *i* in each sampling location *j* within a plot varies as a function of the Julian day (*Day*), with a random intercept (*α*) for each of the seven sampling locations within a plot. With this model, we were able to estimate the LAI values for a given Julian day each year for each treatment (Fig. S5). This in turn allowed us to obtain the Julian days in which LAI starts to increase (positive slope change) from the minimum and when it starts to decrease (negative slope change) after the maximum LAI.

Supplementary Tables

TABLE S1. Plot size, focal species combination, and fertilization scheme (kg) for each fertilizer employed in the experiment. For each plot, we provide the stand species combination: *Dalbergia retusa* Hemsl. (DALRET), *Enterolobium cyclocarpum* (Jacq.) Griseb. (ENTCYC), *Hymenaea courbaril* L. (HYMCOU), *Simarouba glauca* DC. (SIMGLA), *Swietenia macrophylla* King. (SWIMAC), and *Handroanthus impeteginosus* (Mart. ex DC.) Mattos (TABIMP). Experimental treatment abbreviations go as follows: Control, Drought+Fertilizer (D+F), Drought (D), and Fertilizer (F).

Plot ID	Treatment	Stand	Area (m^2)	Basacote® Plus 3M	Osmocote® Plus
1	Control	HYMCOU-DALRET	189.00		
2	D+F	HYMCOU-DALRET	163.68	15.35	16.37
3	Control	HYMCOU-SWIMAC	180.00		
4	D+F	HYMCOU-SWIMAC	134.68	12.63	13.47
5	F	HYMCOU-TABIMP	180.00	16.88	18.00
6	D	HYMCOU-TABIMP	120.00		
7	D	HYMCOU-DALRET	144.00		
8	F	HYMCOU-DALRET	150.00	14.06	15.00
9	D	ENTCYC-DALRET	180.00		
10	F	ENTCYC-DALRET	336.00	31.50	33.60
11	D+F	ENTCYC-TABIMP	326.51	30.61	32.65
12	Control	ENTCYC-TABIMP	357.00		
13	D	SIMGLA-SWIMAC	144.00		
14	F	SIMGLA-SWIMAC	180.00	16.88	18.00
15	Control	SIMGLA-DALRET	189.00		
16	D+F	SIMGLA-DALRET	189.10	17.73	18.91

*Total fertilizer values (kg) that were divided into two applications (June and September) during each year from August 2016 to September 2020.

TABLE S2. Particle size distribution in 2016 (%), extractable elements (Olson extractable Cu, Zn, Mn, Fe, and P in mg kg^{-1}, and K in cmol kg^{-1}), and total C and N (in %) for samples collected in the fifth year (2021). Experimental treatment (TRT) abbreviations go as follows: Control (C), Drought+Fertilizer (D+F), Drought (D), and Fertilizer (F). Stand species combination: *H. courbaril – D. retusa* (HD), *H. courbaril – S. macrophylla* (HS), *H. courbaril – H. impeteginosus* (HT), *E. cyclocarpum – D. retusa* (ED), *E. cyclocarpum – H. impeteginosus* (ET), *S. glauca – S. macrophylla* (SS), and *S. glauca – D. retusa* (SD).

Plot	Stand	TRT	Clay	Silt	Sand	pH	K	P	Cu	Zn	Mn	Fe	C	N
1	HD	C	35.88	38.67	25.44	5.8	0.89	2.3	9.8	3.9	17	57	3.23	0.33
2	HD	D+F	31.39	55.55	22.71	5.4	1.14	15.5	14.6	7.8	28	123	4.44	0.48
3	HS	C	35.18	57.97	20.25	5.9	1.00	2.0	12.9	4.0	21	59	3.57	0.38
4	HS	D+F	33.51	56.53	21.13	5.9	1.31	9.5	14.3	7.3	21	64	4.27	0.43
5	HT	F	31.15	53.53	25.93	5.6	1.21	12.3	16.9	9.0	40	80	4.32	0.46
6	HT	D	33.13	57.35	19.55	6.0	1.01	5.3	13.2	7.0	21	59	4.05	0.42
7	HD	D	31.98	52.87	28.68	5.9	0.84	2.9	14.1	4.5	18	70	3.20	0.38
8	HD	F	35.82	57.1	23.66	5.7	0.99	7.2	15.4	6.3	31	89	3.66	0.39
9	ED	D	33.74	54.96	25.61	6.1	1.17	4.3	11.5	5.6	23	52	3.60	0.39
10	ED	F	31.58	54.59	24.36	5.7	1.61	15.5	13.4	6.3	26	90	3.68	0.38
11	ET	D+F	20.65	47.98	27.3	5.8	1.42	8.7	10.4	4.8	11	79	5.82	0.55
12	ET	C	14.29	41.37	34.02	6.4	1.18	2.6	5.1	3.0	4.9	47	5.97	0.55
13	SS	D	24.74	47.98	28.4	6.4	1.42	3.3	4.4	2.0	7.2	46	5.51	0.42
14	SS	F	23.77	46.92	29.24	6.1	1.04	3.9	6.2	2.3	7.2	56	5.66	0.40
15	SD	C	20.13	54.13	12.07	6.4	1.03	2.2	6.1	1.9	6.6	38	6.45	0.44
16	SD	D+F	27.34	60.59	7.51	5.9	1.27	4.3	10.4	3.9	8.1	68	5.66	0.45

TABLE S3. Chemical composition of the two complete formula slow-release fertilizers used in the fertilization treatments: Fertilizer and Drought+Fertilizer. Where the table shows the percentages of both macro- and micronutrients: nitrogen (N), phosphorus pentoxide (P_2O_5), potassium oxide (K_2O), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mb) and zinc (Zn).

Nutrient	Basacote® Plus 3M	Osmocote® Plus
N	16.00	15.00
P_2O_5	8.00	9.00
K_2O	12.00	12.00
Mg	2.00*	1.00
S	12.00	2.30
B	0.02	0.02
Cu	0.05	0.05
Fe	0.40	0.45
Mn	0.06	0.06
Mo	0.02	0.02
Zn	0.02	0.05

*Added as magnesium oxide (MgO).

TABLE S4. Mean and its associated standard deviation for each productivity flux in a throughfall manipulation plus fertilization experiment in northwestern Costa Rica. Where we show values during four years of experimental manipulation for litterfall annual productivity (PP_L), annual reproductive biomass productivity (PP_F), annual fine roots productivity (PP_R), annual woody productivity (PP_W), and the annual count of nodules. All productivity fluxes are expressed in units of kg m^{-2} yr^{-1}.

Response variable: RGR for plantation trees

Model predictors:	F-value	d.f.	p-value
N-fixer (Yes – No)	**4.1841**	**1**	**0.0512**
Drought (Yes – No)	2.3472	1	0.1489
Fertilizer (Yes – No)	1.1400	1	0.3041
Drought:Fertilizer	**5.1626**	**1**	**0.0499**
N-fixer:Drought	0.0037	1	0.9522
N-fixer:Fertilizer	0.0033	1	0.9546

Response variable: RGR for plantation trees

Model predictors:	F-value	d.f.	p-value
Deciduous (Yes – No)	**3.9466**	**1**	**0.0639**
Drought (Yes – No)	2.5649	1	0.1304
Fertilizer (Yes – No)	0.6986	1	0.4164
Drought:Fertilizer	2.8509	1	0.1217
Deciduous:Drought	0.0154	1	0.9026
Deciduous:Fertilizer	0.2101	1	0.6524

Response variable: RGR for understory trees

Model predictors:	F-value	d.f.	p-value
N-fixer (Yes – No)	**21.1139**	**1**	**0.0001**
Drought (Yes – No)	0.0339	1	0.8601
Fertilizer (Yes – No)	0.2167	1	0.6580
Drought:Fertilizer	**5.0374**	**1**	**0.0659**
N-fixer:Drought	0.8576	1	0.9026
N-fixer:Fertilizer	0.3012	1	0.6524

TABLE S5. The number of dead stems per year and the associated biomass lost (BL) over four years in a throughfall manipulation plus fertilization experiment in northwestern Costa Rica. Numbers highlighted in bold include at least one stem from the 30-yr old plantation which implies a major shift in forest structure (i.e., light, nutrient, and water availability) as these trees represent most of the biomass and canopy cover.

Plot	Treatment	2017	2018	2019	2020	Total	BL (kg m^{-2})
1	Control				5	5	0.09
2	Drought + Fertilizer			5	7	12	0.31
3	Control			1	2	3	0.03
4	Drought + Fertilizer			1		1	0.01
5	Fertilizer	**3**			**1**	4	0.45
6	Drought			1	1	2	0.06
7	Drought				3	3	0.05
8	Fertilizer			3		3	0.03
9	Drought			1	1	2	0.04
10	Fertilizer			2	3	5	0.05
11	Drought + Fertilizer	4		1	**2**	7	1.67
12	Control	**2**		1		3	0.40
13	Drought			2	2	4	0.06
14	Fertilizer			1	2	3	0.06
15	Control			3	**2**	5	0.37
16	Drought + Fertilizer	**1**	**2**		1	4	2.26

Supplementary Figures

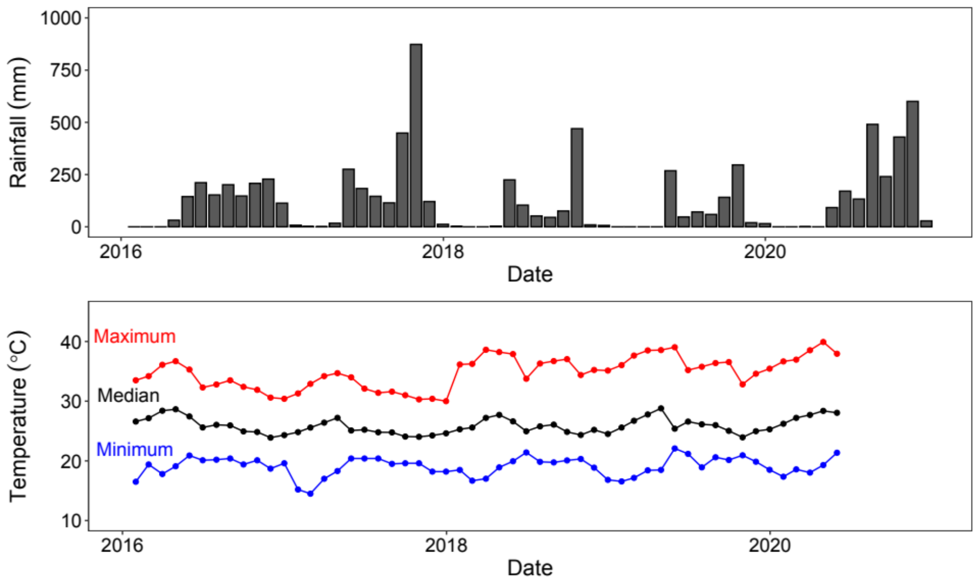

Fig. S1. Monthly rainfall (upper panel) and monthly temperature (lower panel) from a HOBO U30 weather station (Onset Computer Corporation, Bourne, MA, USA) located between 1.9 and 2.5 km from each plot at Estación Experimental Forestal Horizontes, Guanacaste, Costa Rica. From January 2016 to December 2017 and June 2020 to December 2020 the weather station was down, therefore for those two years we complemented our data using a nearby (~14 km) weather station in Santa Rosa National Park, Guanacaste, Costa Rica.

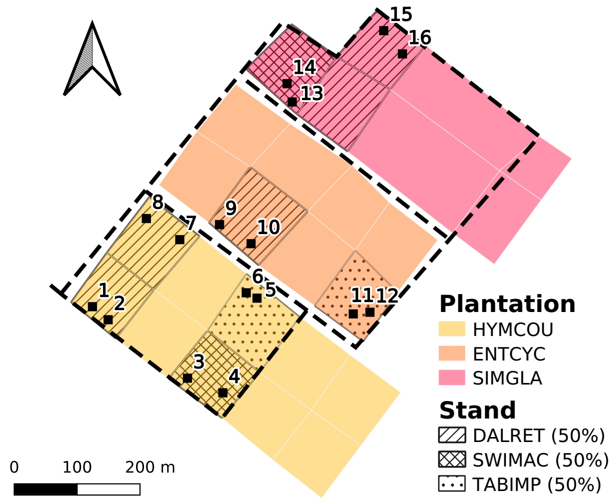

Fig. S2. Layout of the plantations (colored polygons) and the species combination stands (patterned polygons) at Estación Experimental Forestal Horizontes, Guanacaste, Costa Rica. From a total of 11 species, we selected six focal tree species: *D. retusa* Hemsl. (DALRET), *E. cyclocarpum* (Jacq.) Griseb. (ENTCYC), *H. courbaril* L. (HYMCOU), *S. glauca* DC. (SIMGLA), *S. macrophylla* King. (SWIMAC), and *H. impeteginosus* (Mart. ex DC.) Mattos (TABIMP). Non-patterned polygons represent additional combination of species not included in this study. Dashed lines represent trails and black squares the location of the experimental plots.

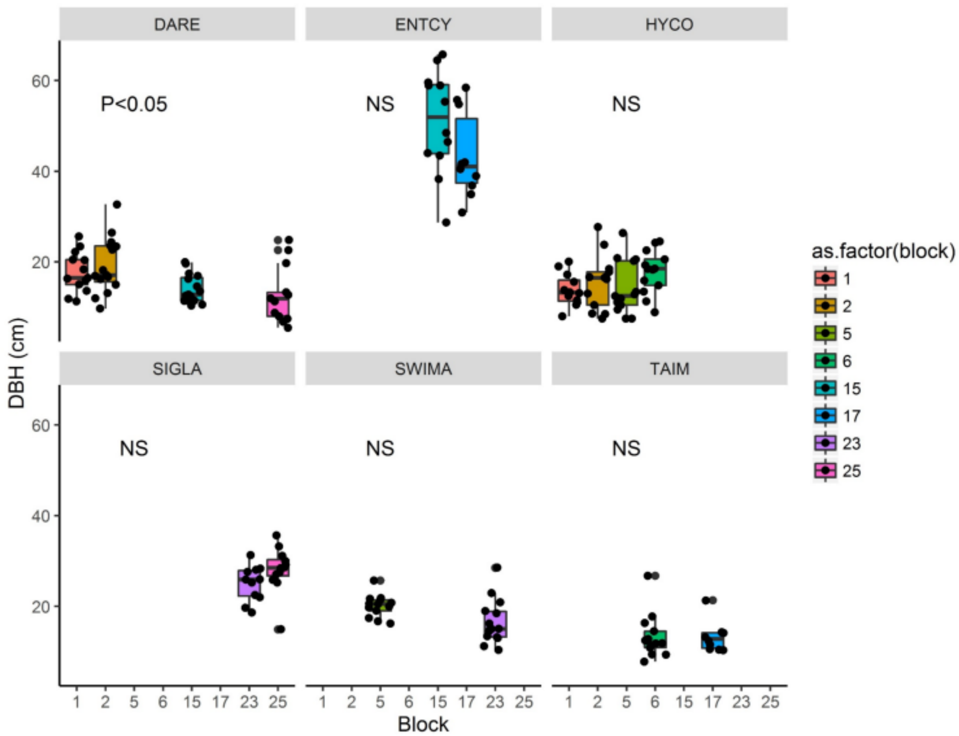

Fig. S3. Diameter at breast height (DBH) for the plantation blocks selected to establish the rainfall manipulation and fertilization experiment in Northwestern Costa Rica. We selected six focal tree species to perform the experiment: *D. retusa* Hemsl. (DARE), *E. cyclocarpum* (Jacq.) Griseb. (ENTCY), *H. courbaril* L. (HYCO), *S. glauca* DC. (SIGLA), *S. macrophylla* King. (SWIMA), and *H. impeteginosus* (Mart. ex DC.) Mattos (TAIM). Significance obtained from species-specific analyses of variance to test the effect of the plantation block on the DBH.

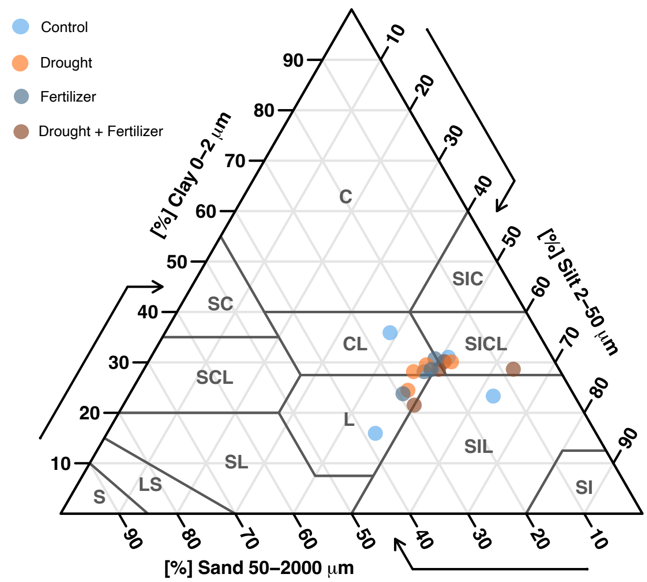

Fig. S4. Soil particle size distribution for each experimental treatment in this study. Classification triangle according to the United States Department of Agriculture: clay (C), silty clay (SIC), sandy clay (SC), clay loam (CL), silty clay loam (SICL), sandy clay loam (SCL), loam (L), silty loam (SIL), sandy loam (SL), silty (SI), loamy sand (LS) and sand (S).

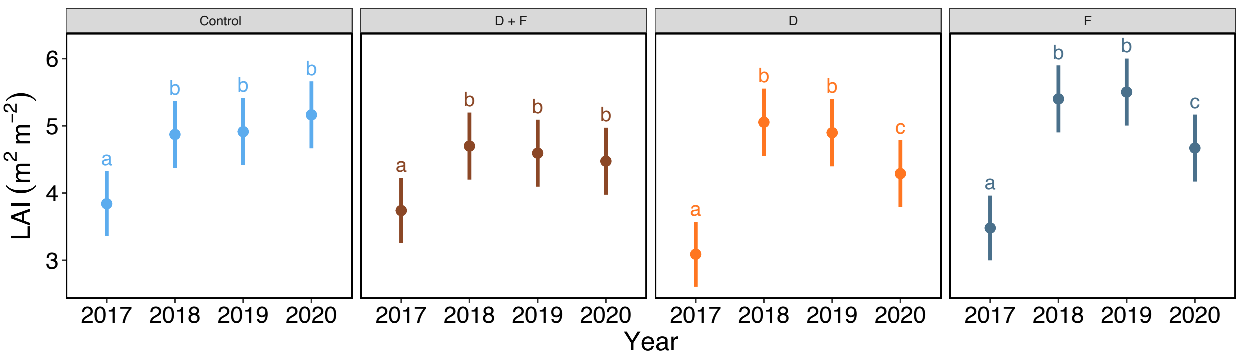

Fig. S5. Leaf area index mean values with 95% confidence intervals for each experimental treatment (Control, D+F: Drought+Fertilizer, D: Drought, F: Fertilizer) during the month of November. We found a significant year effect ($F=21.29$; d.f.= 3; $p < 0.001$) because of tropical storm Nate, in which LAI decreased during November 2017 across all treatments. Letters represent multiple comparisons among years within experimental treatments from a Post-Hoc Tukey's honest significance difference test.

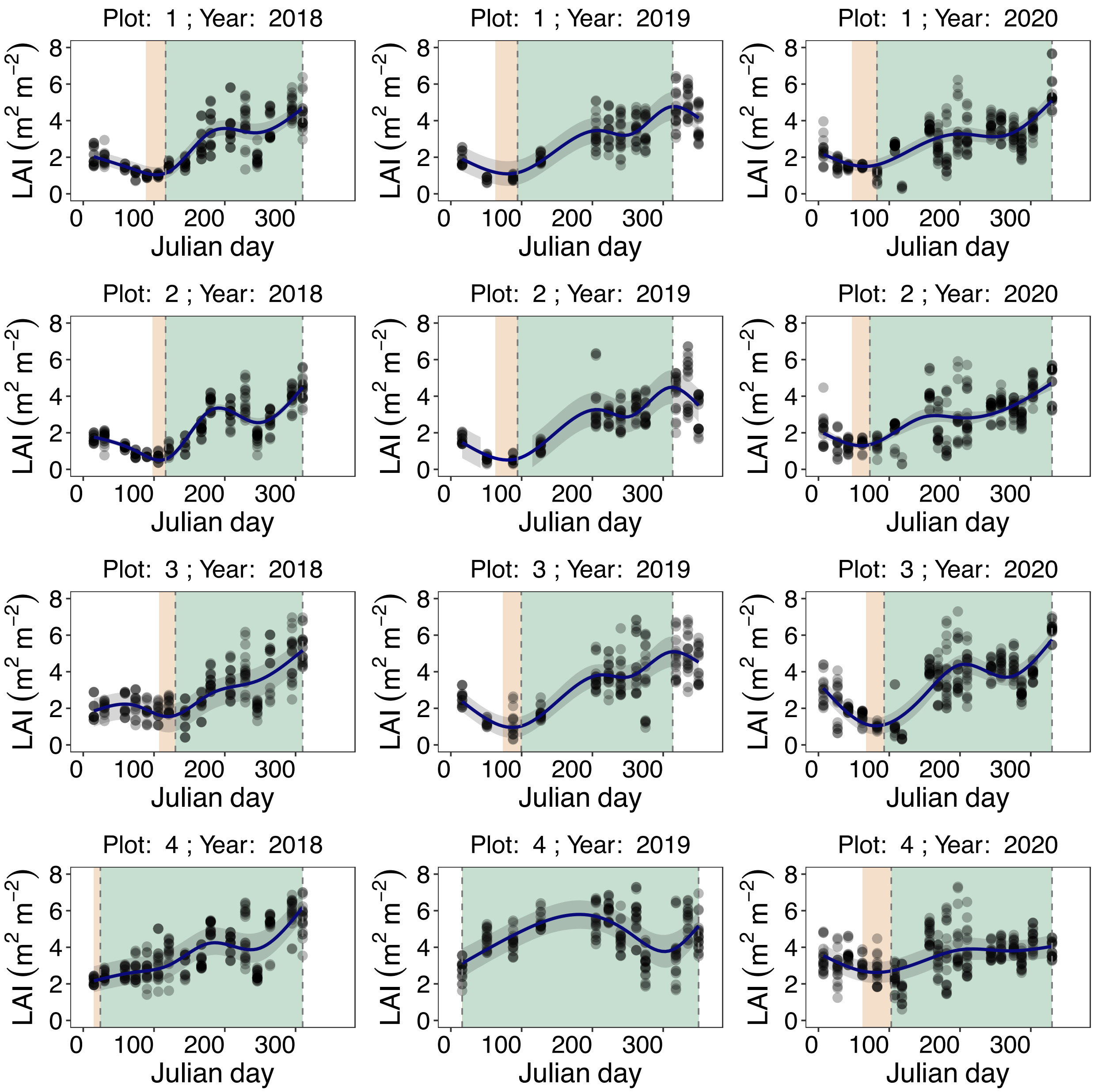

Fig. S6. Scatter-plots of leaf area index (LAI) as a function of the Julian day per year showing plot-specific general additive model cubic regression spline functions with 95% confidence intervals used to calculate LAI-derived metrics. In orange the period where minimum LAI was maintained (slope = 0) and in green the period in which LAI was increasing or the growing season (slope > 0).

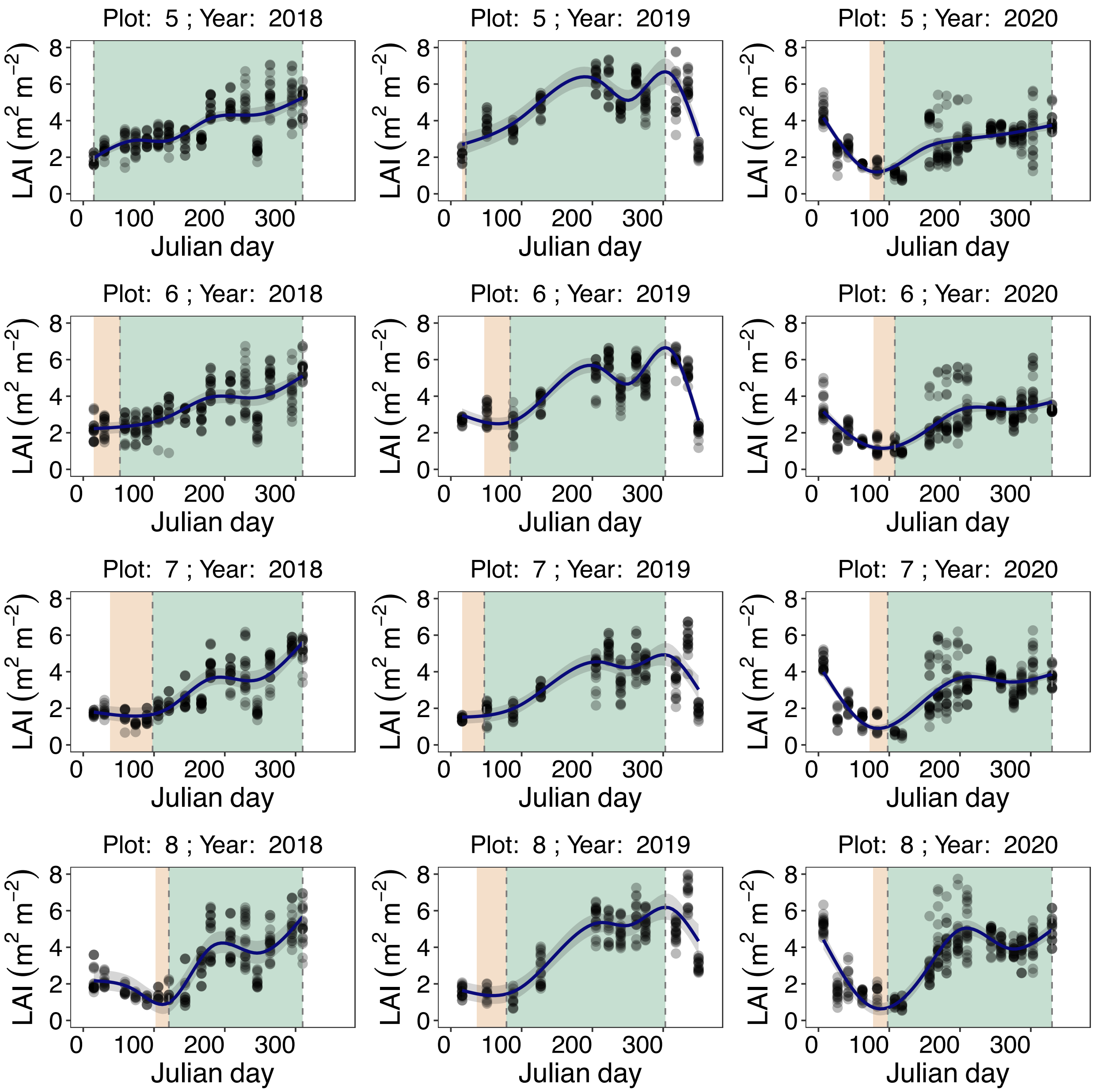

Fig. S6. (continued) Scatter-plots of leaf area index (LAI) as a function of the Julian day per year showing plot-specific general additive model cubic regression spline functions with 95% confidence intervals used to calculate LAI-derived metrics. In orange the period where minimum LAI was maintained (slope = 0) and in green the period in which LAI was increasing or the growing season (slope > 0).

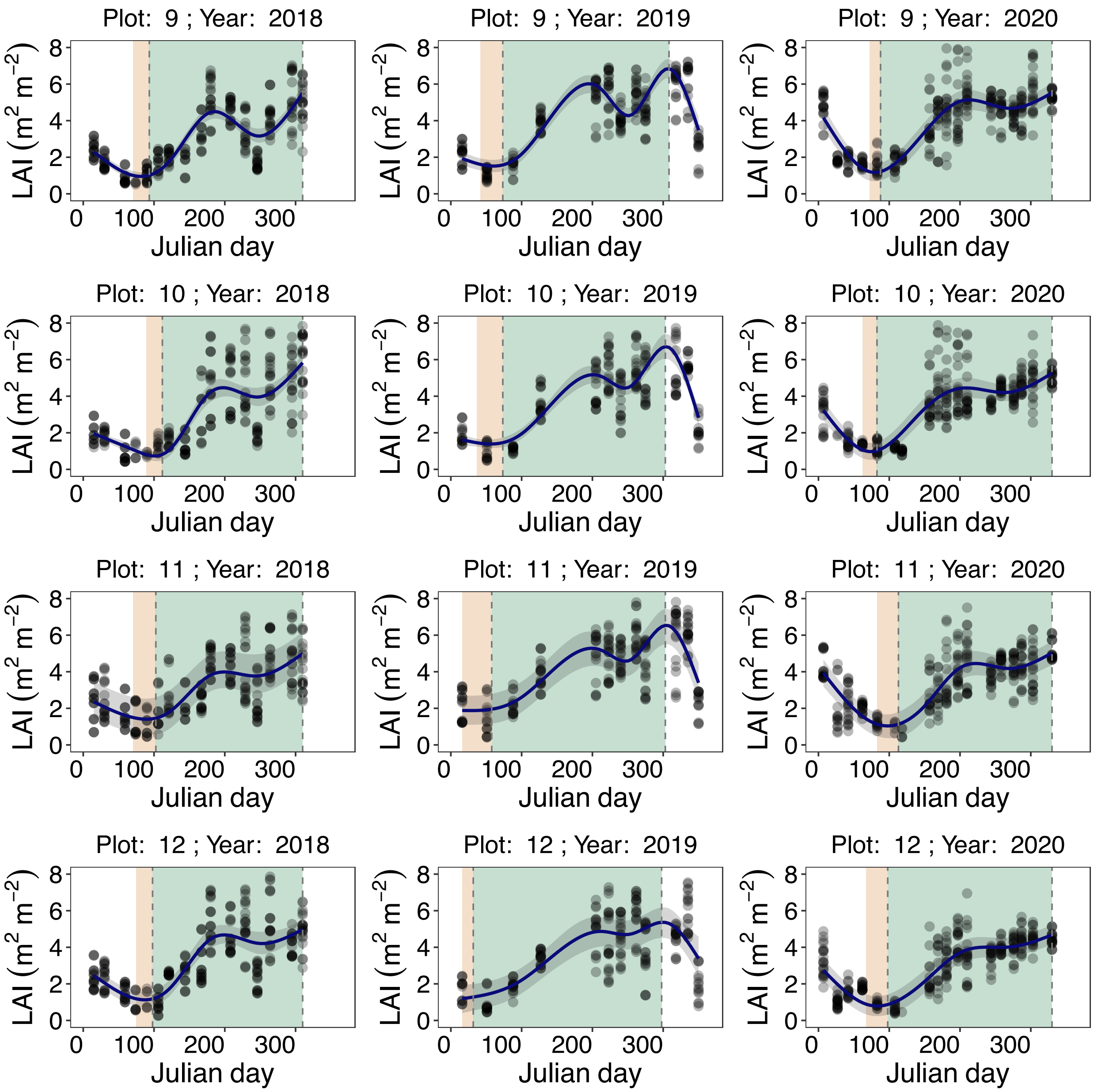

Fig. S6. (continued) Scatter-plots of leaf area index (LAI) as a function of the Julian day per year showing plot-specific general additive model cubic regression spline functions with 95% confidence intervals used to calculate LAI-derived metrics. In orange the period where minimum LAI was maintained (slope = 0) and in green the period in which LAI was increasing or the growing season (slope > 0).

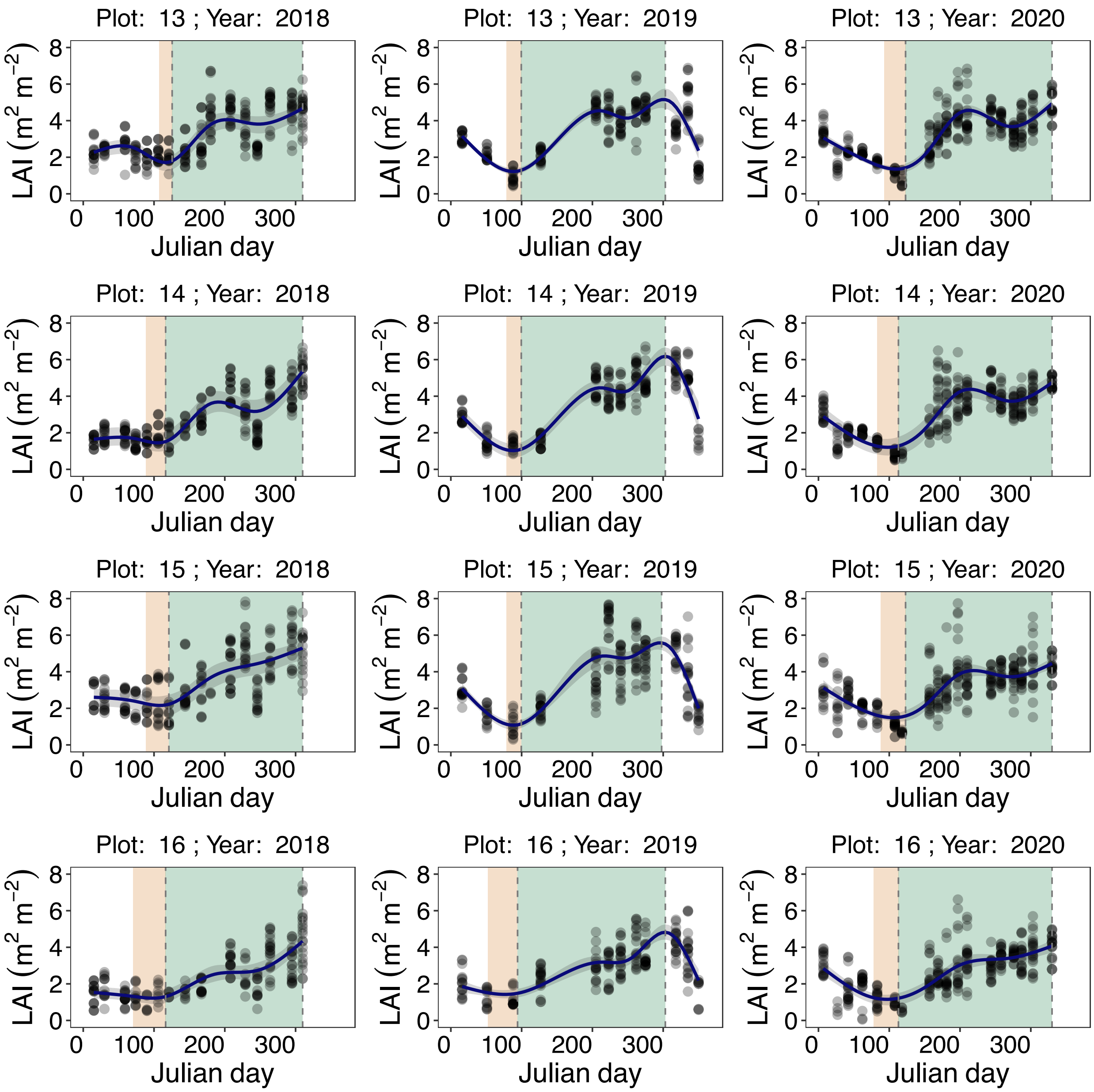

Fig. S6. (continued) Scatter-plots of leaf area index (LAI) as a function of the Julian day per year showing plot-specific general additive model cubic regression spline functions with 95% confidence intervals used to calculate LAI-derived metrics. In orange the period where minimum LAI was maintained (slope = 0) and in green the period in which LAI was increasing or the growing season (slope > 0).

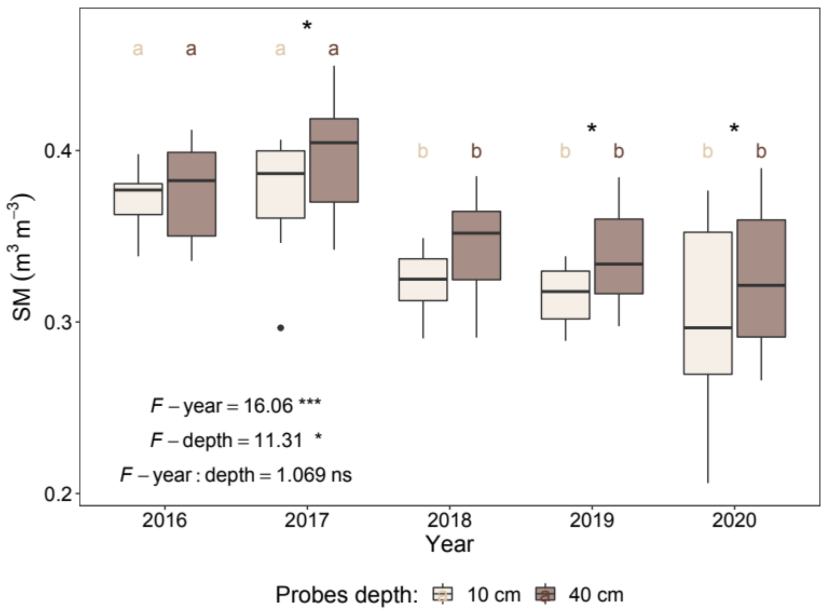

Fig. S7. Volumetric water content (SM) during the wet season for plots without a throughfall exclusion structure. Reported results from a two-way repeated-measures analysis of variance for the median wet season SM in four years. Lowercase letters stand significant differences within depths among years, and asterisks significant differences between depths within years. In both cases, we performed pairwise comparisons using a Post-Hoc Tukey's honest significance difference test.

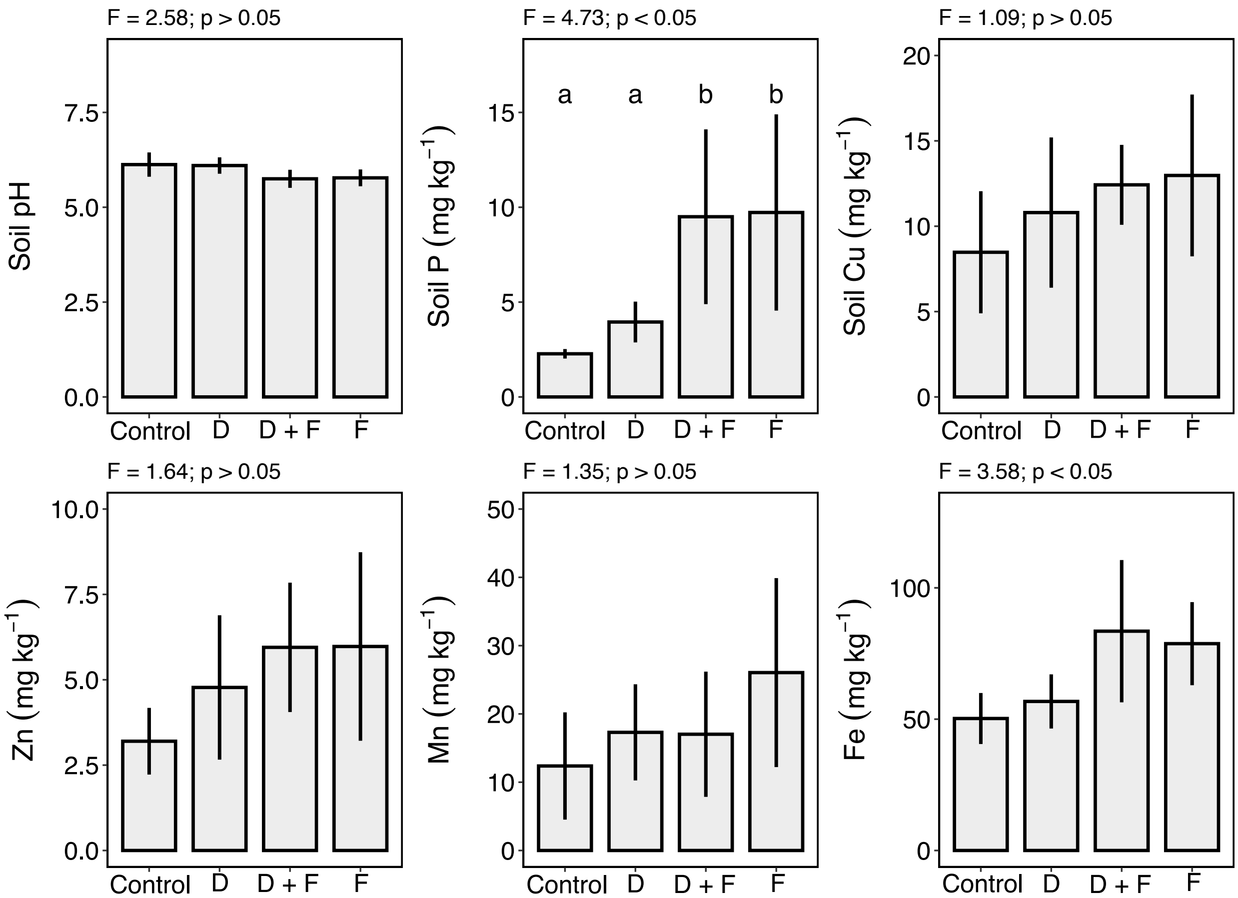

Fig. S8. Soil pH and Olson extractable elements composition in the four experimental treatments: control, drought (D), drought+fertilizer (D + F), and fertilizer (F). Reported results from a one-way analysis of variance for soils collected in 2021, after four years of nutrient additions. Lowercase letters stand for multiple comparisons among experimental treatments from a Post-Hoc Tukey's honest significance difference test.

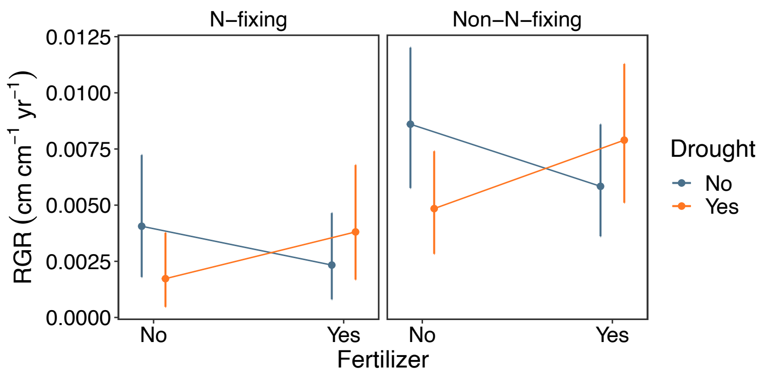

Fig. S9. Relative growth rates (RGR) for plantation trees. Mean plots with their associated standard error show the interaction (*F* = 5.16, *d.f.* = 1, ***p* = 0.0499**) between the effects of fertilizer and drought treatments on nitrogen-fixing (N-fixing) and non-nitrogen-fixing (Non-N-fixing) plant functional types.

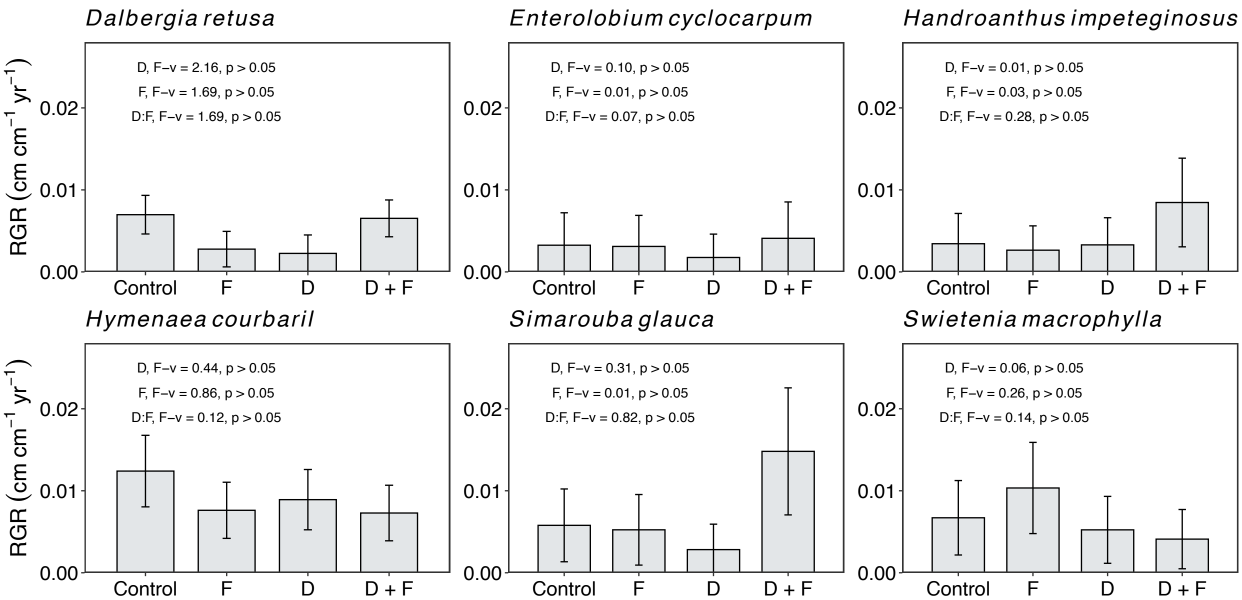

Fig. S10. Diameter at breast height relative growth rates (RGR) over four years as a function of the experimental treatments: control, drought (D), drought+fertilization (D + F), and fertilization (F). Bar-plots showing the media, the standard deviation (error bars), and reported results from a two-way analysis of variance for each of the six focal tree species in the plantations.

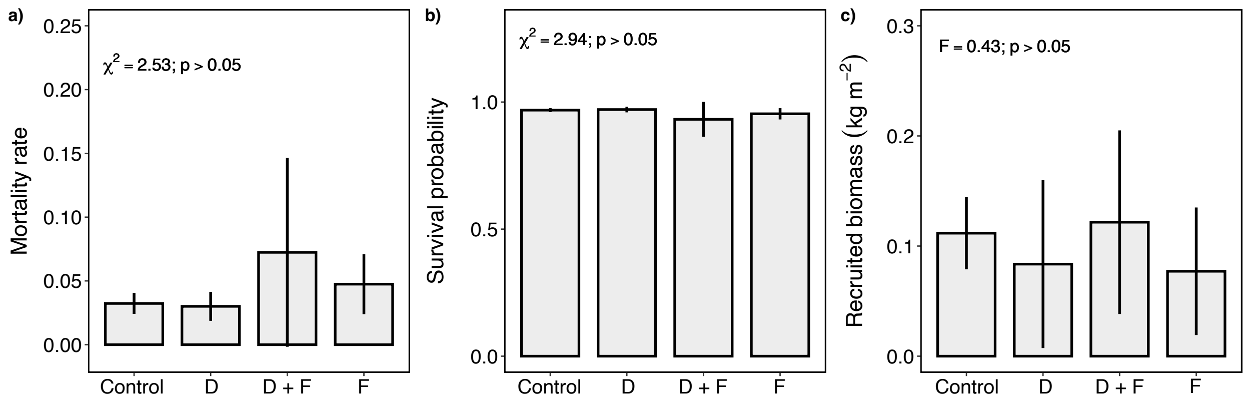

Fig. S11. Demographic rates in a four-year interval (2016-2020) for understory individuals defined as the trees with a diameter at breast height (DBH) higher than 2.5 cm and lower than 7.5 cm as a function of the experimental treatments: control, drought (D), drought+fertilization (D + F), and fertilization (F). We calculated the survival probability (a) and the mortality rate (b) as Condit et al. (2006). The recruitment rate (c) was calculated as the biomass of the total number of stems per plot area that recruited in the time interval.

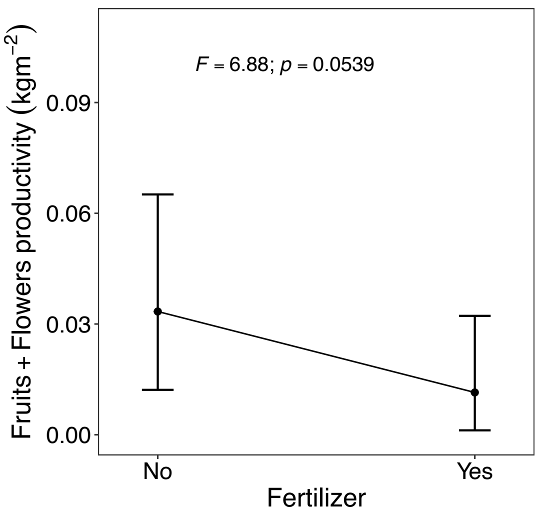

Fig. S12. Productivity of reproductive structures as a function of the fertilization treatment. Results from a two-analysis of variance with stand, plot, and sampling unit as random effects.

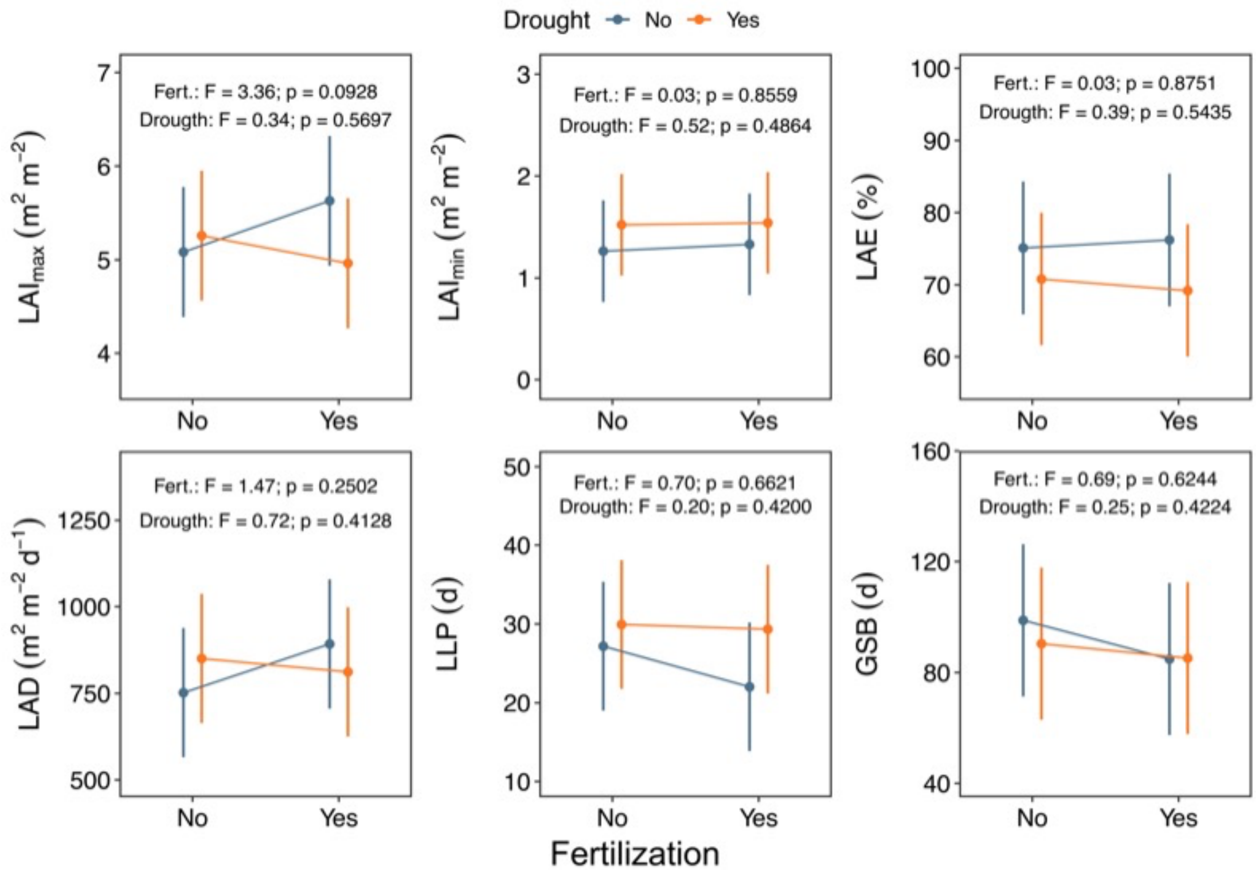

Fig. S13. Responses of leaf area index (LAI) metrics to drought and fertilization treatments. Mean plots showing the media, the standard error (error bars), and reported results from a two-way analysis of variance for each of the six LAI-derived metrics: maximum LAI (LAI_{max}), minimum LAI (LAI_{min}), leaf area enlargement (LAE), leaf area duration (LAD), leafless period (LLP), and the start of the leaf flushing (GSB).

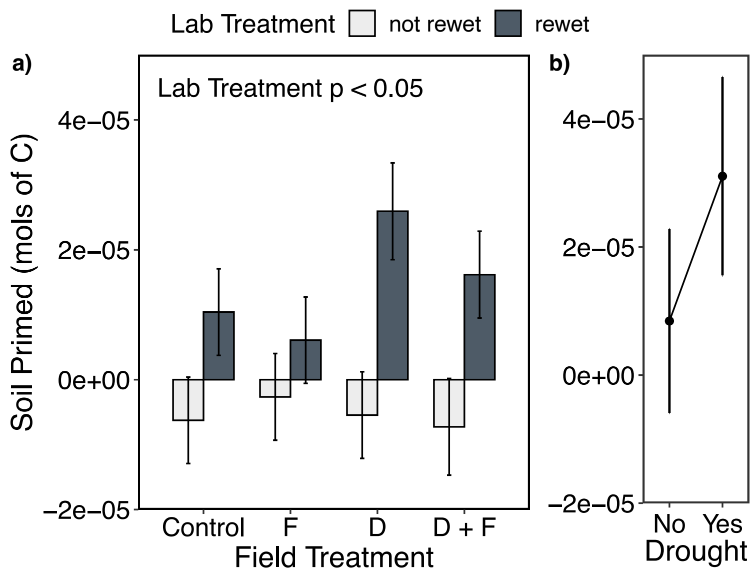

Fig. S14. a) Responses of priming to two laboratory treatments using soils from each field treatments: control, fertilization (F), drought (D), and drought+fertilization (D + F). b) Rewet priming response to drought led to higher priming of soil carbon in soils from drought treatment relative to soils with no drought treatment (*F-v* = 5.33, *d.f.* = 1, ***p-v* = 0.0497**).

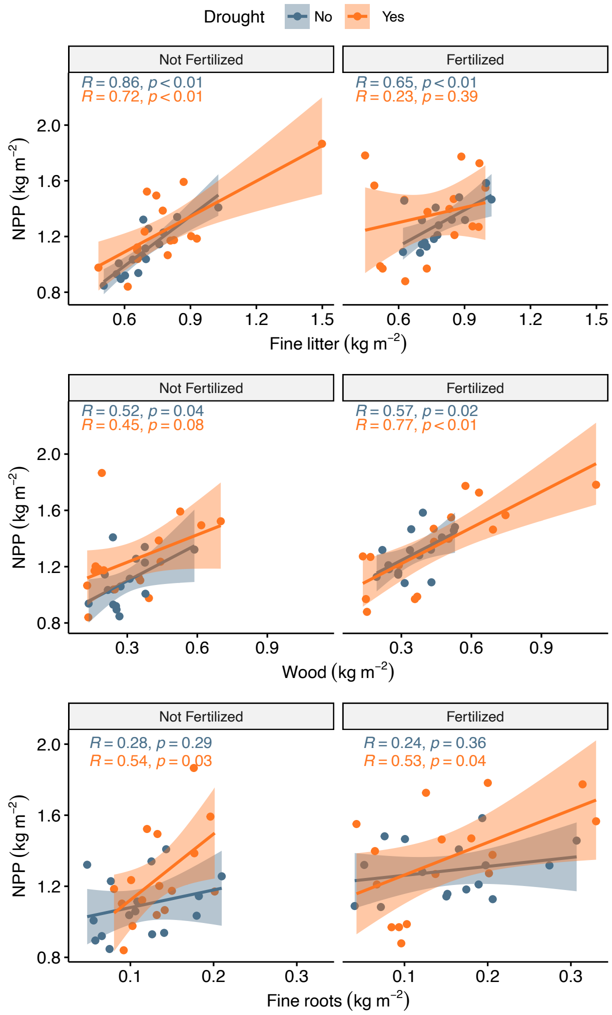

Fig. S15. Pearson correlation coefficients (R) and their associated significance for net primary productivity (NPP) as a function of each biomass flux for each experimental treatment.

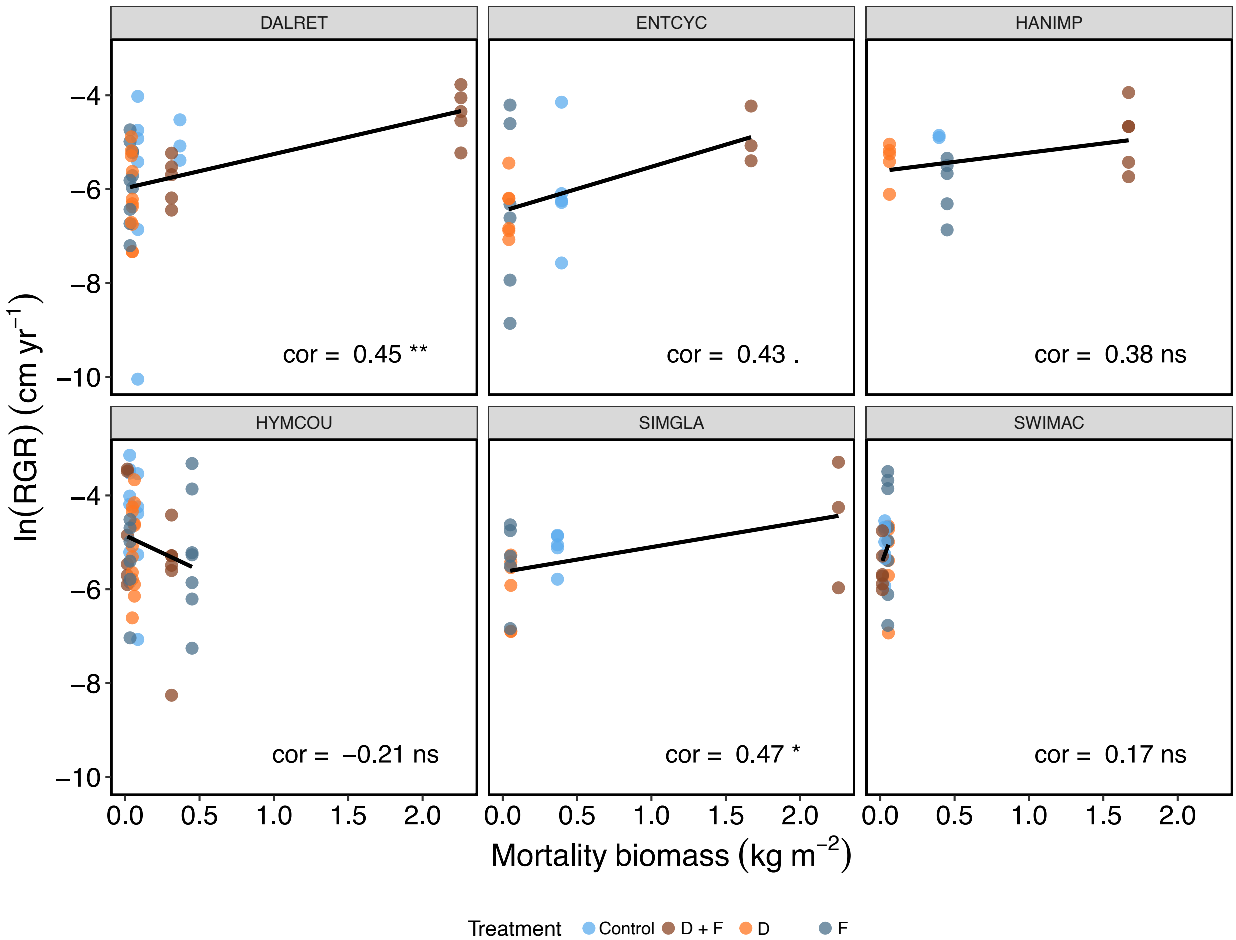

Fig. S16. Individual diameter at breast height relative growth rates (RGR) over four years as a function of the biomass loss due to mortality (Mortality biomass) for a given plot. Each dot represents an individual tree from the six focal species: *D. retusa* (DALRET), *E. cyclocarpum* (ENTCYC), *H. impeteginosus* (HANIMP), *H. courbaril* (HYMCOU), *S. glauca* (SIMGLA), and *S. macrophylla* (SWIMAC). Colors stand for experimental treatments such as control, drought+fertilization (DF), drought (DR), and fertilization (FR). Pearson's product-moment correlation (cor) and the associated significance level at: $p > 0.10$ (ns), $p > 0.05$ (.), $p < 0.05$ (*), and $p < 0.01$ (**).

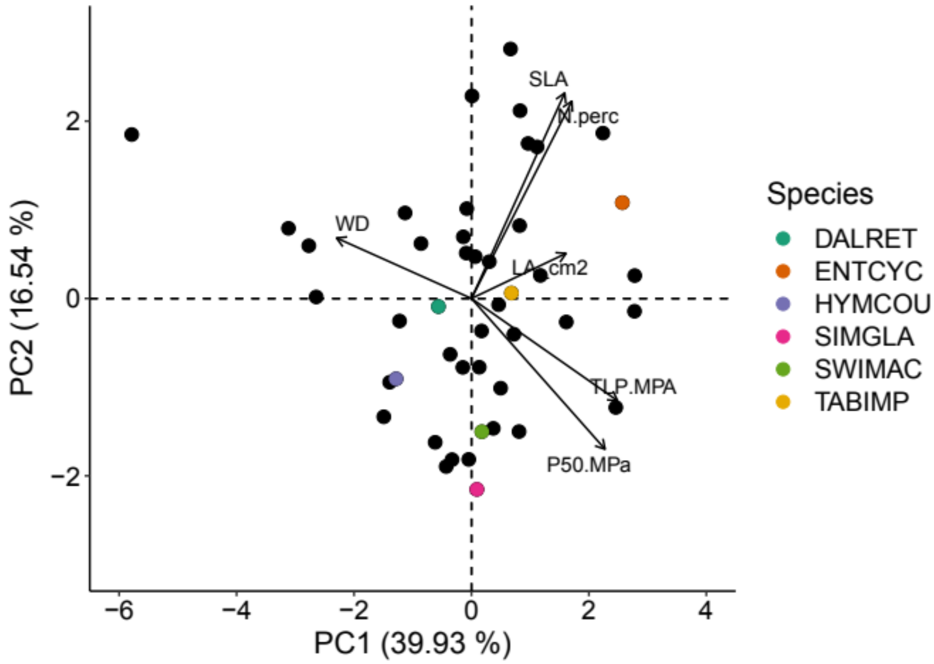

Fig. S17. Bi-plot of the first two axes from a principal components analysis (PCA) describing the hydraulically safe-risky (PC1) and the fast-slow (PC2) plant economic spectrum. Each dot represents a species from the tropical dry forest biome and colors correspond to the focal tree species in this study: *D. retusa* (DALRET), *E. cyclocarpum* (ENTCYC), *H. impeteginosus* (HANIMP), *H. courbaril* (HYMCOU), *S. glauca* (SIMGLA), and *S. macrophylla* (SWIMAC). Plant functional traits are defined as: leaf area (LA.cm2), specific leaf area (SLA), foliar nitrogen content (N.perc), wood density (WD), the water potential at 50% accumulation of leaf veins embolisms (P50.MPa), and the water potential at turgor loss point (TLP.MPA). Figure adapted from Vargas G. et al. (2021).

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