New Phytologist Supporting Information

Article title: **African Savanna grasses outperform trees across the full spectrum of soil moisture availability**

Authors: Michael W. Belovitch, Julienne E. NeSmith, Jesse B. Nippert and Ricardo M. Holdo

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Table S1 Sample sizes for tree and grass species used for analyses of whole-plant transpiration (WPT), leaf turgor loss point (TLP), whole-plant water use efficiency (WUE) and relative growth rate (RGR).

FT	Family	Species & Auth.	Code	WPT	TLP	WUE	RGR
Grass	Poaceae	*Aristida adscensionis* L.	Ariads	-	4	-	-
Grass	Poaceae	*Aristida congesta* Roem &Schult	Aricon	3	5	-	2
Grass	Poaceae	*Aristida congesta barbicollis* Trin & Rupr	Aribar†	2	-	-	-
Grass	Poaceae	*Aristida stipitata* Hack.	Aristi	1	-	-	-
Grass	Poaceae	*Aristida meridionalis* Henrard	Arimer	3	-	-	2
Grass	Poaceae	*Brachiaria deflexa* Schumach.	Bradef	-	5	-	-
Grass	Poaceae	*Cenchrus ciliaris* L.	Cencil	-	5	-	-
Grass	Poaceae	*Chloris virgata* Sw.	Chlvir	3	5	-	3
Grass	Poaceae	*Cynodon dactylon* (L.) Pers.	Cyndac	-	5	-	-
Grass	Poaceae	*Digitaria eriantha* Steud.	Digeri	3	4	-	-
Grass	Poaceae	*Eleusine Africana* Kenn. -O'Bryne	Eleafr	-	5	-	-
Grass	Poaceae	*Enneapogon cenchroides* C.E.Hubb	Enncen	-	5	-	-
Grass	Poaceae	*Eragrostis gummiflua* Nees	Eragum	3	-	-	3
Grass	Poaceae	*Eragrostis lehmanniana* Nees	Eraleh	-	5	-	-
Grass	Poaceae	*Eragrostis superba* Peyr.	Erasup	3	5	3	3
Grass	Poaceae	*Eragrostis trichophora* Hochst.	Eratri	3	5	-	3
Grass	Poaceae	*Hyperthelia dissoluta* (Nees) Clayton	Hypdis	-	-	4	-
Grass	Poaceae	*Melinis repens* Zizka	Melrep	3	4	-	1
Grass	Poaceae	*Megathyrsus maximus* Jacq.	Panmax	3	5	5	3
Grass	Poaceae	*Perotis patens* Gand.	Perpat	3	5	5	3
Grass	Poaceae	*Pogonarthria squarrosa* Pilg.	Pogsqu	3	5	4	3
Grass	Poaceae	*Schmidtia pappophoroides* J.A.Schmidt	Schpap	3	-	-	1
Grass	Poaceae	*Setaria sphacelata* Stapf & C.E.Hubb	Setsph	3	-	-	1
Grass	Poaceae	*Sporobolus nitens* Stent	Sponit	-	5	-	-
Grass	Poaceae	*Themeda triandra* Forssk.	Thetri	3	5	-	2
Grass	Poaceae	*Tricholaena monachne* Stapf & C.E.Hubb	Trimon	3	-	-	3
Grass	Poaceae	*Trichoneura grandiglumis* Ekman	Trigra	3	-	-	3
Grass	Poaceae	*Urochloa mosambicensis* (Hack.) Dandy	Uromos	3	5	-	3
Tree	Fabaceae	*Vachellia exuvialis* Kyal & Boatwr	Acaexu	3	4	-	3
Tree	Fabaceae	*Vachellia gerrardii* Benth.	Acager	3	5	-	3

Tree	Fabaceae	*Vachellia grandicornuta* Gerstner	Acagra	2	-	-	-
Tree	Fabaceae	*Vachellia luederitzii* Kyal. & Boatwr	Acalue	-	4	-	-
Tree	Fabaceae	*Vachellia nigrescens* P.J.H.Hurter	Acanig	3	5	-	2
Tree	Fabaceae	*Vachellia nilotica* P.J.H.Hurter & Mabb.	Acanil	-	5	-	-
Tree	Fabaceae	*Vachellia tortilis* Galasso & Banfi	Acator	2	4	-	-
Tree	Fabaceae	*Vachellia xanthophloea* P.J.H.Hurter	Acaxan	3	-	-	3
Tree	Fabaceae	*Albizia harveyii* E.Fourn.	Albhar	3	5	-	2
Tree	Fabaceae	*Albizia versicolor* Welw. ex Oliv.	Albver	1	1	-	-
Tree	Fabaceae	*Bauhinia galpinii* N.E.Br.	Baugal	-	5	-	-
Tree	Rhamnaceae	*Berchemia discolor* Hemsl.	Berdis	3	-	-	3
Tree	Fabaceae	*Brachystegia spiciformis* Benth.	Braspi	3	-	-	1
Tree	Fabaceae	*Colophospermum mopane* Benth. Leonard	Colmop	3	5	8	2
Tree	Combretaceae	*Combretum apiculatum* Sond.	Comapi	3	5	2	3
Tree	Combretaceae	*Combretum collinum* Fresen.	Comcol	3	4	-	-
Tree	Combretaceae	*Combretum hereroense* Schinz	Comher	2	5	-	-
Tree	Combretaceae	*Combretum imberbe* Wawra	Comimb	-	5	-	-
Tree	Combretaceae	*Combretum zeyheri* Sond.	Comzeh	3	-	-	2
Tree	Fabaceae	*Crotalaria natalitia* Meissner	Cronat	-	5	-	-
Tree	Fabaceae	*Dalbergia melanoxylon* Guill. & Perr.	Dalmel	1	5	-	-
Tree	Fabaceae	*Dichrostachys cinerea* Wight & Arn.	Diccin	1	5	8	-
Tree	Ebenaceae	*Diospyros mespiliformis* Hochst. Ex A.DC.	Diomes	2	5	-	-
Tree	Malvaceae	*Dombeya rotundifolia* Planch.	Domrot	3	-	-	-
Tree	Ebenaceae	*Euclea divinorum* Hiern	Eucdiv	1	5	-	-
Tree	Ebenaceae	*Euclea natalensis* A.DC.	Eucnat	-	4	-	-
Tree	Moraceaea	*Ficus sycomorus* L.	Ficsyc	-	2	-	-
Tree	Malvaceae	*Grewia bicolor* Gaertn.	Grebic	-	5	-	-
Tree	Celastraceae	*Gymnosporia senegalensis* Loes.	Gymsen	-	3	-	-
Tree	Capparaceaea	*Maerua parvifolia* Pax	Maepar	-	5	-	-
Tree	Fabaceae	*Peltophorum africanum* Sond.	Pelafr	3	8	-	1
Tree	Fabaceae	*Philenoptera violacea* (Klotzsch) Schrire	Phivio	2	3	-	-
Tree	Fabaceae	*Pterocarpus angolensis* DC.	Pteang	-	5	-	-
Tree	Fabaceae	*Pterocarpus rotundifolius* (Sond.) Druce	Pterot	1	5	-	-
Tree	Fabaceae	*Schotia brachypetala* Sond.	Schbra	3	-	-	3
Tree	Anacardiaceae	*Sclerocarya birrea* Hochst.	Sclbir	3	5	10	3
Tree	Loganiaceae	*Strychnos madagascariensis* Poir.	Strmad	3	5	-	-
Tree	Combretaceae	*Terminalia sericea* Mart.	Terser	-	7	1	-
Tree	Rubiaceae	*Vangueria infausta* Burch.	Vaninf	-	4	-	-
Tree	Rhamnaceae	*Ziziphus mucronata* Willd.	Zizmuc	-	5	-	-

[†]Subspecies of *A. congesta*

Fig. S1 Six plants at the start of a run of growth chamber measurements. The top and bottom of all pots were sealed with cling film (Glad® Press'n Seal®), and aluminum foil. Plants were placed on top of scales, which logged mass every 5 min.

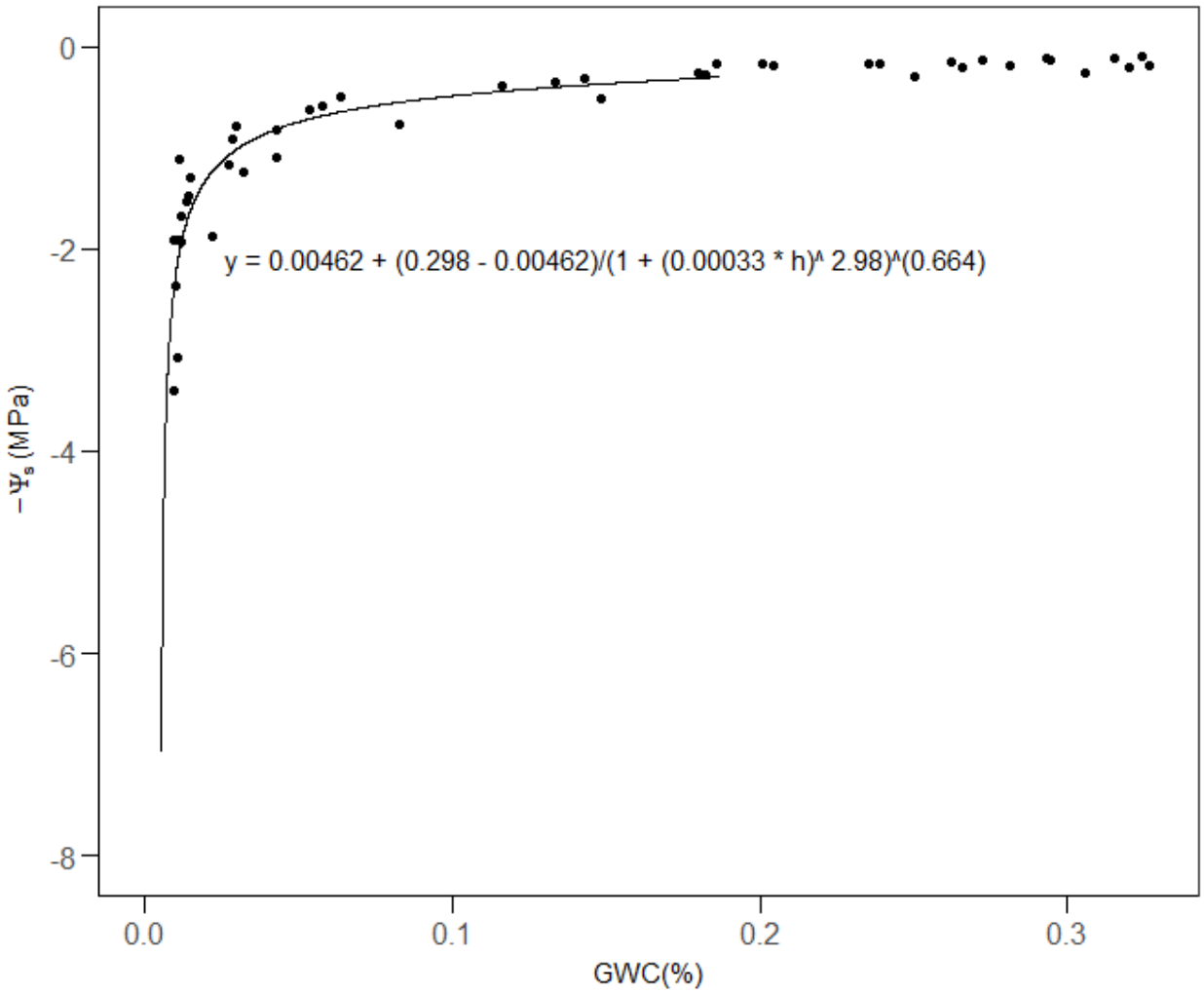

Fig. S2 Soil water retention curve calculated using a gravimetric approach for this study's soil. We fit a van Genuchten function (van Genuchten, 1980) to the data (N = 48 measurements, adj. $R^2 = 0.92$) and used it to transform our GWC values into Ψ_S time series for each pot.

Methods S1 Supplementary methods describing whole plant transpiration measurements, water use efficiency (WUE) and relative growth rate (RGR) measurements.

After germinating seedlings in seed trays, we transplanted tree and grass seedlings (median transplant dates for grasses and trees were 3 and 10 Jun 2019, respectively) into 4.5 l pots filled with sand. Our choice of soil medium was intended to reflect the prevalent sandy soil conditions prevalent at WRF. We applied Banrot fungicide to all pots. We also applied 22.5 cm^3 of slow-release fertilizer (Osmocote 14-14-14) and 1.25 cm^3 each of gypsum and ground dolomitic lime over our medium. We conducted whole-plant transpiration measurements in a growth chamber (Percival E-41HO, Percival Scientific, Perry, IA) at the Odum School of Ecology between 6 Jun and 4 Dec 2019 (Fig. S1). We used a gravimetric method based on sealing plants and pots to prevent water loss through leakage or evaporation (Holdo & McHargue, 2020). We wrapped each pot with cling film (Glad® Press'n Seal®), taking care to secure the film as tightly as possible around the base of each plant, and added a layer of aluminum foil around the base of the plant to prevent leakage. We placed pots on individual scales (Ohaus Scout Pro, Ohaus Corp., Parsippany, NJ), and considered any mass loss recorded (to a precision of 0.1 g) to represent plant transpiration, given that the rate of plant mass gain through C assimilation is negligible relative to water loss. We transferred six individuals at a time in randomized batches to the growth chamber. Before starting a batch of measurements, we estimated and then added the amount of water needed to bring a given pot to a well-watered state (with plant transpiration near its maximum) while preventing leakage through the bottom of the pot. This meant that pots varied somewhat in terms of their initial soil water potential.

We set the growth chamber to a 12:12 h day: night cycle at 30/25°C, respectively. We kept a plant in the growth chamber until it was visibly beginning to wilt (mean and SD = 8.1 and 4.5 d, respectively), ensuring that our transpiration measurements spanned a wide range of water potentials. A weather station datalogger (Kestrel 5000 Environmental Meter, Nielsen-Kellerman, Boothwyn, PA) within the growth chamber recorded ambient temperature and relative humidity every 5 minutes. Given the variation in height across individuals, and the decay in light intensity as a function of distance to the light source at the top of the growth chamber, we wanted to ensure that differences in transpiration patterns between functional types or across species would not be confounded with differences in incidental light regimes (McAusland et al., 2016). Prior to insertion into the chamber, therefore, we photographed each plant in the laboratory against a white background to reconstruct the vertical profile of its photosynthetic biomass. We photographed the plants with a Nikon D90 SLR (Nikon Inc., Tokyo, Japan) at high resolution, using a 5-cm scale in the photo frame to convert pixels to cm. We cropped the photos to the extent of each plant and then used an R script to automate the process of extracting green pixels (corresponding to photosynthetic surfaces) and generating height profiles of green cross-sectional area in 5-cm height bins.

At harvest, we collected, oven-dried (48 h at 60 °C) and weighed all aboveground biomass, which we separated into leaves and stems. To reconstruct the soil water potential time series experienced by a plant during its time in the growth chamber, we combined a gravimetric approach with a water retention curve calibrated for the soil we used in the study. Prior to sealing each pot before insertion into the chamber, we used a metal tube (~ 2 cm diameter) to collect two soil samples from opposite ends of each pot, ensuring that the sample was representative of the entire vertical profile of the soil within the pot. At harvest time, we emptied the contents of each pot onto a tray and mixed the soil before collecting two final soil samples. We weighed the four

soil samples (two initial and two final) immediately upon collection to obtain wet mass, and again following drying for 48 h at 105 °C. We then calculated mean initial and final gravimetric water content (GWC) for each pot and used changes in pot mass over time to build GWC time series for each pot. To convert GWC to soil water potential (Ψ_S), we constructed a water retention curve for the soil we used in the greenhouse. We placed samples of ~ 5 g dry soil in five separate stainless-steel cuvettes and used a pipette to add enough deionized water to saturate the samples. Starting from this saturated condition, we allowed the samples to progressively air-dry, obtaining paired GWC (through weighing) and Ψ_S measurements at regular intervals. To measure Ψ_S, we used a WP4C dewpoint potentiometer (Meter Group, Inc.).

For the measurement of whole-plant WUE, we planted seeds in Feb/Mar 2020, and subsequently transplanted them into 4.5 l pots filled with sand. We watered all pots to saturation twice-weekly, and in Aug 2020 we fertilized the pots with 5 cm^3 of Osmocote 14-14-14. Growing conditions were comparable to those reported for the transpiration experiment. Beginning in Aug 2020, we watered all pots to field capacity and sealed the pots with cling film, adding aluminum foil to the pot bases to prevent leakage. We recorded initial pot masses and used these as target masses for the experiment, periodically adding water to each pot to keep each plant well-watered. Over the next six weeks, we recorded pot mass pre- and post-watering every three days, which allowed us to track cumulative plant water consumption. To calculate plant growth over this period, we used a combination of photography and biomass harvests. At the beginning and end of the experimental period, we took cross-sectional photographs of each individual plant to quantify green cross-sectional area using the imaging methods described above. In this case we placed each plant on a turntable and rotated it 360° in 45° increments for a series of eight photographs. We used mean cross-sectional area across the photo series as an index of shoot biomass. We then regressed the harvest biomass (obtained by oven-drying harvested shoots) against the harvest cross-sectional area to infer the initial shoot biomass, for which we did not have actual biomass data. Given the low sample sizes within species, we used a two combined regression models, one for trees and one for grasses. The regressions suggested that the photographic method produces a reasonable estimate of shoot mass (adjusted $R^2 = 0.92$ for trees and 0.63 for grasses).

Unlike the case for the whole-plant transpiration and WUE measurements, for the RGR measurements we planted seeds in a sand medium to allow soil removal from roots prior to transplantation. This allowed us to accurately measure initial plant mass for subsequent RGR calculations. Following the emergence of the first true leaf, we rinsed each seedling to remove soil, patted it dry with a paper towel, and then weighed it on an analytical balance to obtain initial wet mass. We then transplanted three seedlings of each species into 4.5 l pots filled with soilless media (Fafard 3B, Sun Gro Horticulture, Agawam, MA). We oven-dried and weighed the remaining seedlings to calculate wet:dry mass ratios. In several cases, low germination rates or transplant failure (attributable to our need to plant seeds in sand rather than growing medium) led to lower sample sizes than our target. For the transplanted seedlings, we followed the fertilization and watering regimes used in the transpiration study. At the end of the study, we washed, dried and weighed all plants for calculation of final dry mass (M_{final}). Harvest dates varied by species and were dictated by a target height equivalent to the height of the pots (40 cm). We used initial wet mass and the wet:dry mass ratio per species to estimate initial dry mass ($M_{initial}$).

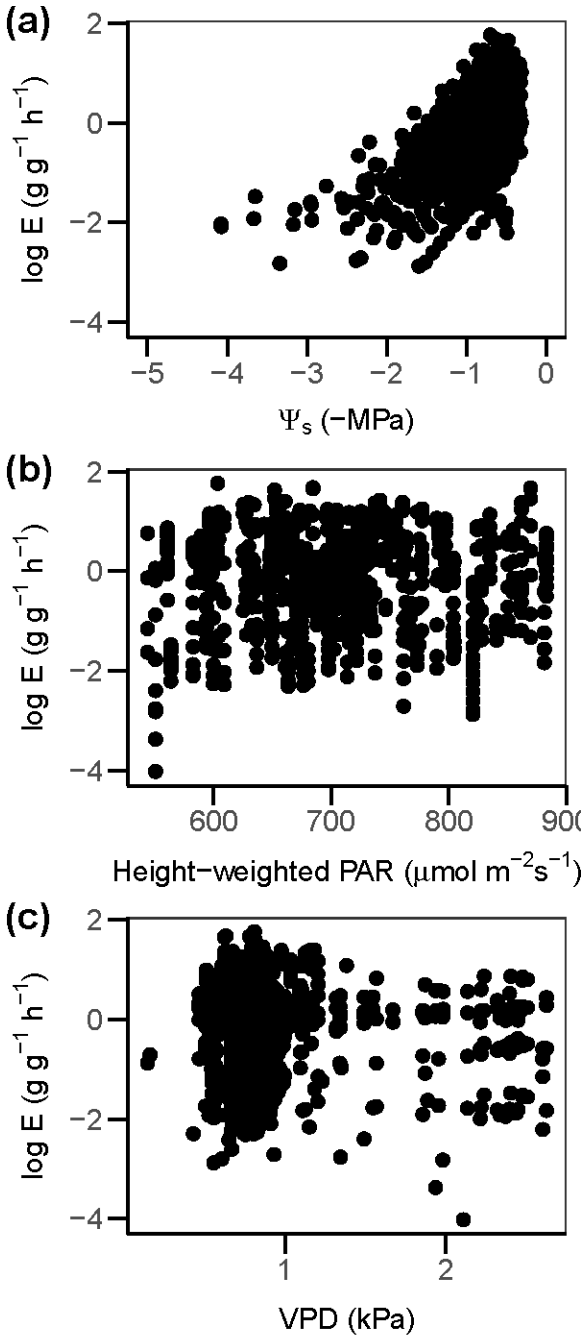

Fig. S3 Hourly transpiration (normalized by leaf mass) as a function of (a) soil water potential (Ψ_S), (b) incident radiation weighted by plant biomass distribution as a function of height, and (c) vapor pressure deficit (VPD) for savanna tree seedlings and grasses under growth chamber conditions. The x-axis in panel a has been truncated below -5 MPa to increase legibility.

Table S2 Model fits (given by AIC, the Akaike Information Criterion) for four models of hourly transpiration (normalized by leaf mass) for savanna tree seedlings and grasses under growth chamber conditions. In all cases, species, batch (corresponding to a run of six plants), day in chamber and plant ID were treated as random effects.

Model fixed effects	Random effect σ				Residual σ	df	AIC†
	Species	Batch	Day	ID			
Null	0.21	0.41	0.56	0.49	0.44	6	1777.3
VPD	0.20	0.47	0.57	0.49	0.44	7	1771.5
VPD + Radiation	0.15	0.49	0.57	0.50	0.44	8	1771.3
Ψ_S	0.28	0.44	0.26	0.32	0.31	7	1032.7

†The model with the lowest AIC has the strongest support

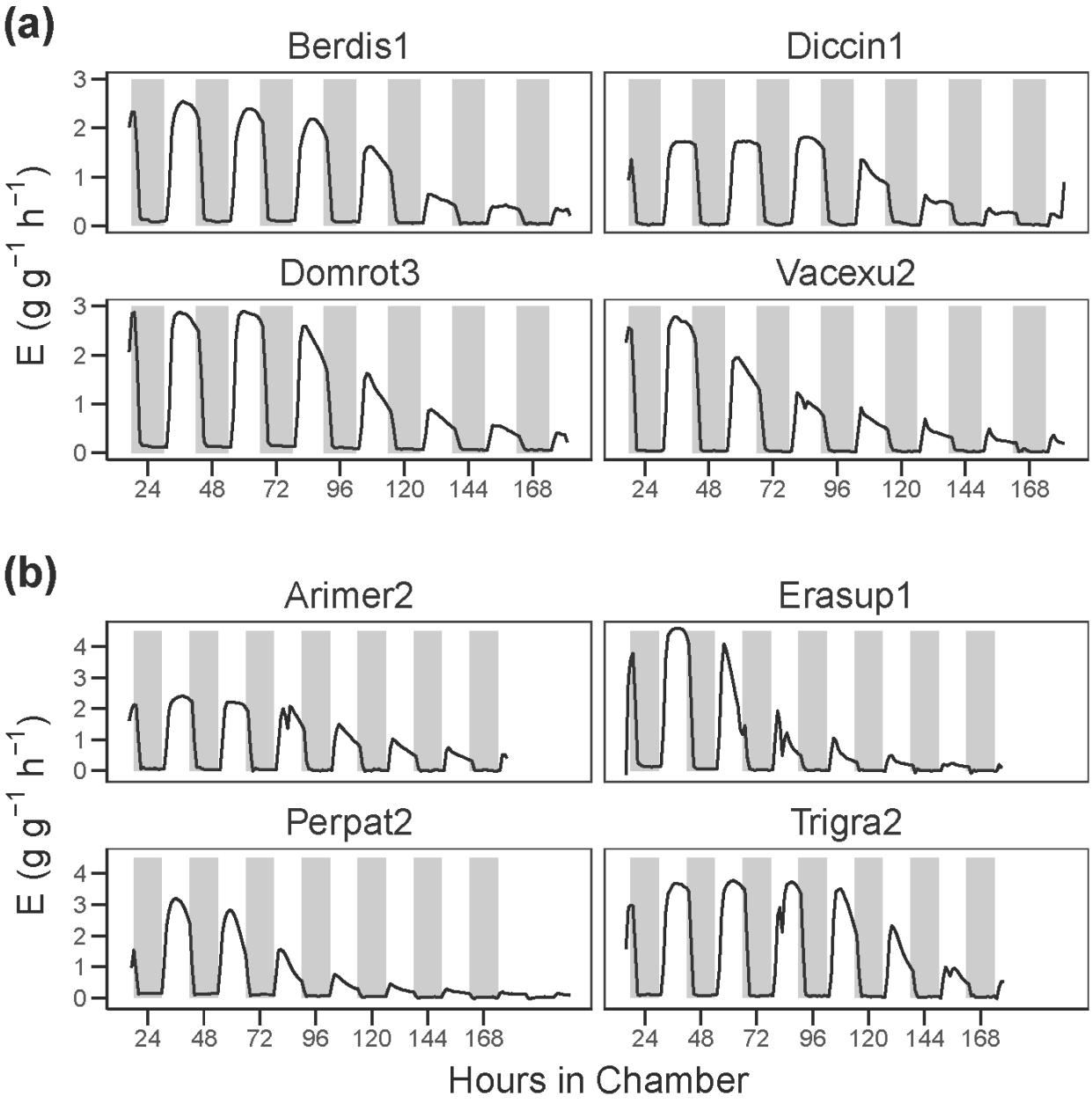

Fig. S4 Hourly transpiration time series (normalized by leaf mass) for (a) four savanna tree seedlings and (b) four grass tussocks under growth chamber conditions. Shaded regions represent dark (12-h) periods. Individual plant codes represent species codes (Table S1) + replicate for a given species.

References

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McAusland, L., Vialet-Chabrand, S., Davey, P., Baker, N.R., Brendel, O. and Lawson, T. (2016), Effects of kinetics of light-induced stomatal responses on photosynthesis and water-use efficiency. New Phytol, 211: 1209-1220. https://doi.org/10.1111/nph.14000