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Consistent trade-offs in fungal trait expression across broad spatial scales

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SUPPLEMENTARY METHODS

Exploring the dispersion of functional traits within communities necessitates that those traits are measured within individual species from the community¹. However, the hyper-diverse and opaque nature of fungal communities presents a number of challenges for the measurement of individual-level functional traits *in situ*. Specifically, it is often challenging to allocate trait expression to any given individual species within a community. As a result, fungal traits are often estimated either using molecular approaches that explore species-level gene expression that might potentially relate to individual-level trait expression, or by directly measuring the characteristics of individuals growing in axenic culture conditions. Although both approaches have limitations that restrict the reliability of trait estimates relative to the expression of those traits *in situ*, they can provide valuable information about the fundamental principles and trade-offs governing fungal activity in general.

Here, we estimate direct trait expression from fungal isolates growing under optimal, axenic conditions, following greenhouse studies in plant communities. Therefore, although the trait estimates in our study are unlikely to represent the realized trait expression by those individuals growing under sub-optimal, natural conditions, the energetic and biochemical restrictions on fungal activity will lead to equivalent underlying patterns in trait expression across a range of species. All fungal isolates were obtained from the USDA Forest Service, Center for Forest Mycology Research (CFMR) culture collection, maintained by the Northern Research Station and located at the Forest Products Laboratory, Madison, Wisconsin (USA). They were maintained within 2% (w/v) MEA in 10-cm diameter Petri dishes. Each isolate had originally been collected from fruiting bodies in early-to-mid stage dead wood (decay stages 1-2.5) in mixed forests. The selected fungi were intended to represent a wide phylogenetic range, including considerable within and between species variation, and were originally collected from across a climatic gradient across the North American continent.

(Please see references ²⁻⁴ for additional methods, as many of the methods given below are summarized from these sources.)

Growth rate

Fungi were inoculated into each plate by placing a 5-mm diam. plug of previously colonized MEA onto the centre of each plate using a sterilized cork borer. Linear hyphal extension rate, relative to the inoculum plug, was measured daily for two weeks, or until the isolate reached the edge of the plate. Growth rate was calculated as the average distance per day^{5,6}. Each assay was replicated five times per isolate.

For temperature response curves, each fungus was grown at 10, 16, 22, 28, 34, and 40 °C, replicated five times for each temperature. For moisture potential, MEA plates were amended

with potassium chloride (KCl) to form a gradient of matric potential ⁷⁻⁹. Specifically, 2% MEA was amended with 0, 2.7, 5.4, 10.8, 16.1 and 21.5g of KCl per 300 mL of MEA, yielding approximate matric potentials of -0.5, -1.0, -1.5, -2.5, -3.5, and -4.5 MPa, respectively. The relationships between KCl and MPa was taken from previous research ¹⁰, and extrapolated to more negative MPa values. As noted in the main text, these values should thus be seen as approximate. Each fungus was replicated three times at each water potential values.

Moisture and temperature growth curves and niche widths

Motivated by the approach of Lennon et al. ¹¹, we used nonlinear least squares to estimate the growth curves from the experimental data. Due to the shapes of the raw curves, we chose to use a standard 3-parameter skew normal distribution to fit the data. For each fungus, we used quasi-Newton optimization in the *optim* function in R to find the set of parameters that minimized the sum of squares between the observed growth rates and the best-fitting skew normal distribution (using the *dsn* function in R). This process was conducted separately using the temperature and moisture growth data. The niche width was calculated using the result growth curves, and estimated as the range of temperature and moisture values for which growth is at least one-half their maximum growth rates¹¹.

Density

Following previous methods^{12,13}, mycelia were collected from each dish by overlaying the MEA with cellophane (Fischer Scientific, NC9823382), which allows for uptake of nutrients and water, but prevents mycelia from burrowing into the agar. Cellophane disks (10-cm diam.) were cut, submerged in a flask of DI water, and autoclaved for 5 min. A sterile disk was placed in each plate and isolates were inoculated as above. Plates were incubated at 22°C for two weeks, or until the isolate reached the edge of the plate. Subsequently, a 1 cm x 1 cm square of colonised cellophane was cut from each plate at a distance of 1 cm from the edge of the growing front. The squares were dried at 60°C and weighed. The dried mycelia were scraped off each square using a small aluminium spatula, and the cellophane square was re-weighed. The density (dry mass per cm²) was calculated as the average difference in the mass of the cellophane squares before and after removing the mycelia. Each assay was replicated five times per isolate.

Volatile organic compounds

Volatile organic compounds were sampled using solid adsorbent cartridges analysed using mass spectrometry (see ^{14,15}). Specifically, each fungal isolate was plated onto standard MEA and allowed to grow to a diameter of 5 cm. The plate was then transferred to a glass chamber fitted with a suspended volatile sampling cartridge, containing two adsorbent compounds (Tenax GR and Carbonex 1016). The chambers were sealed for 48 hours, at which point the cartridge was

removed and kept at 4°C until analysis. Volatile organic compound production was analysed using thermodesorption-gas chromatography with flame ionization and mass spectrometry^{14,15}. The instrument background and fluctuations were removed, and a series of negative controls and experimental controls (agar plates with no fungi, blank controls) were used to remove non-fungal VOCs. Compound identification was based on the NIST/EPA/NIH Mass Spectral Library, Version 2.0 of April 2009; retention indices calculated based on the retention times of normal alkanes (carbon numbers n=5 to 16) were used to verify the identifications. Compounds were classified if they had a measured retention index within +/-20 of those found in the NIST database, and thus compound identification should be interpreted as 'probable matches'.

Enzymes

Enzyme analysis of fungal cultures was conducted following previously established methods^{16,17}. Isolates were cultured for seven days, or until they reached a minimum diameter of 2 cm. Four circular agar plugs (7 mm diam. each, ~1.5 cm² total) were taken from 1 cm behind the growing front, replicated four times for each isolate. These plugs were added to 40 mL of 50-mM sodium acetate buffer solution (pH=5.0) in plastic centrifuge tubes. Tube contents were homogenised for 30 seconds and agitated for 2 h at 4°C under constant mixing. The filtered extracts were used for enzyme assays (five hydrolytic and three oxidative enzymes). The hydrolytic enzymes leucine aminopeptidase (LAP), cellobiohydrolase (CBH), acid phosphatase (PHOS), N-acetyl- β -glucosaminidase (NAG), and β -glucosidase (BG) were measured using a 7-amido-4-methylcoumarin (AMC)-linked substrate L-leucine (LAP), and the methylumbelliferyl- (MUB) linked substrates β -D-cellobioside, phosphate, N-acetyl- β -D-glucosaminide, and β -D-glucopyranoside, respectively. Activities of the two phenol oxidases (phenox1 and phenox2) and one peroxidase (perox1), were assayed using the substrates L-3,4-dihydroxyphenylalanine (L-DOPA, 25mM), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS, 10mM), and L-DOPA+H₂O₂ (0.3% hydrogen peroxide), respectively. Enzyme potential was assessed using fluorescence (hydrolytic enzymes) and absorbance (perox1, perox2, phenox1) using previously published methods¹⁸.

Competitive outcomes

Each competitive microcosm was constructed as above, using 2% MEA in deep-well 10-cm diam. Petri dishes. Following previous methods¹⁹, three 5-mm diam. plugs were inoculated onto one side of the dish, 1 cm apart in a straight line, approx. 2 cm from the centre of the dish. By using three plugs rather than a single plug, fungi were able to form a relatively straight and uniform hyphal front across one half of the plate, preventing fast-growing fungi from circumventing the other fungus and immediately acquiring more territory and a larger resource base¹⁹. Using baseline growth rate measurements of the fungi in monoculture, the slower-growing fungus in each pairing was plated proportionately before the faster-growing fungus so

that they were projected to be approx. 5 mm from the centre of the dish (10 mm apart) at the start of the experiment. Since fungi can inhibit growth by volatile production and the secretion of anti-fungal compounds through the substrate²⁰⁻²³, this 1-cm distance between fungi at the start of the study allowed for fungi to participate in some degree of ‘combat-at-a-distance’, while also minimizing the resource-capture rate advantage for the faster growing fungi.

After both fungi were inoculated in each plate, the microcosms were sealed and incubated at 22°C for up to 8 weeks. Plates were tracked until one fungus completely displaced the other or until no displacement was observed for 3 weeks. Plates were checked daily for the first week, and twice-weekly thereafter. The location of each fungus at each date was marked on the bottom of each dish and measurements were taken at the end of the study. Plates remained sealed throughout the study to prevent moisture loss. Competitive outcomes were scored as a win, loss, or draw.

A total of 23 unique fungal species were used in the competition assays. All fungi were competed against all other fungi, giving 253 unique species-by-species competitions. Instead of pseudo-replication (i.e., replicating the same microcosm using identical isolates), we used true replication using multiple isolates of the same species. One species had eight unique isolates (*Armillaria gallica*) and seven species had two unique isolates (*Armillaria tabescens*, *Hyphoderma setigerum*, *Merulius tremellosus*, *Phlebiopsis flavidoalba*, *Phellinus acerina*, *Phlebia rufa*, *Schizophyllum commune*). All isolates were competed against each other, resulting in 637 unique isolate-by-isolate interactions (omitting intraspecific competitions). Each unique species pairing was thus replicated, on average, >2 times, either by replication of one species or by replication of both species across different isolates. Plates where species did not directly interact (i.e., did not meet and showed no signs of retreat or gains in territory, n=22) were discarded from the statistical analyses, giving 615 total microcosm observations.

Offensive and defensive capability were calculated for each fungus by taking the average ability of each fungus to inhibit the growth of their competitor (defensive), versus their ability to continue to overgrowth their competitor (offensive). Specifically, offensive ability for fungus was calculated as its average overgrowth rate, divided by its monoculture growth rate. A value of one thus means that a fungus grows completely unimpeded, with identical extension rate in mixtures as in monoculture; whereas a value of zero means that it stops (or is overgrown) when competing. Pairings where an isolate lost were set equal to zero (no offensive overgrowth). Defensive ability was calculated as the average rate that a fungus is overgrowth, divided by the monoculture growth rate of its *competitor* (rather than itself). Thus, a value of one means that, on average, every competitor overgrew the fungus without any reduction in its growth rate. A value of zero means that every competitor stopped growing completely. Pairings where the isolate overgrew its competitor were set equal to one (i.e., the competitor cannot have a negative growth rate).

Supplemental References

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Supplementary Table 1. The basic performance traits measurements and geographical locations of the 37 fungal isolates.
Only competitive rank is scaled to [0,1], with all other values reported in their respective units. .

Isolate	Latitude	Longitude	Comp. rank rank	Extension rate (mm day ⁻¹)	Hyphal Density (ug cm ²)	Moisture niche width (-MPa)	Temp niche width (°C)	Optimal Temp (°C)	Optimal Moisture (-MPa)
Armillaria gallica FP102531 C6D	30.465247	-89.040298	0.23	0.25	0.1	3.01	24.7	25	0.89
Armillaria gallica EL8 A6F	43.402553	-88.70347	0.16	0.35	1.02	2.08	13.3	29.8	0.78
Armillaria gallica FP102534 A5A	30.465247	-89.040298	0.23	0.21	0.16	3.71	24.4	25.1	0.92
Armillaria gallica FP102535 A5D	30.465247	-89.040298	0.23	0.25	0.5	4.3	24.3	25.8	0.85
Armillaria gallica FP102542 A5B	31.105861	-88.930435	0.37	0.25	0.65	2.57	18.2	23.9	0.9
Armillaria gallica HHB12551 C6C	43.557132	-89.641414	0	0.49	0.91	4.15	16.2	25.7	0.68
Armillaria gallica OC1 A6E	44.886161	-87.859583	0.16	0.25	0.55	2.36	15.4	29.9	0.84
Armillaria gallica SH1 A4A	43.773821	-87.718678	0.4	0.76	0.61	2.04	9.3	26.2	0.41
Armillaria sinapina PR9	46.517665	-86.410071	0.37	0.77	0.12	1.74	16	26.4	0.36
Armillaria tabescens FP102622 A3C	41.022359	-80.648965	0.37	0.5	0.07	3.43	18.2	27.6	0.62
Armillaria tabescens TJV93 261 A1E	29.946494	-90.126196	0	1.07	0.63	1.32	15.8	24.7	0.27
Fomes fomentarius TJV93 7 A3E	43.227069	-89.723847	0.28	4.71	0.002	1.19	9.3	27.3	0.24
Hyphodontia crustosa HHB13392 B7B	60.734518	-146.69118	0.57	1.96	0.12	1.19	23.2	25.6	0.23
Hyphoderma setigerum HHB12156 B3H	43.557132	-89.641414	0.75	4.11	0.09	1.19	12.5	26.8	0.26
Hyphoderma setigerum FP150263 B2C	18.181083	-76.725883	0.57	4.7	0.03	1.38	9.7	26.7	0.56
Laetiporus caribensis GDL1 A1A	16.256538	-61.784048	0.52	3.77	0.1	1.85	12.7	27.5	0.65
Laetiporus conifericola HHB15411 C8B	49.39377	-123.19093	0.28	5.16	0.04	1.22	12.1	27.1	0.56
Lentinus crinitus PR2058 C1B	18.298306	-65.777922	0.57	6.38	0.05	1.55	17.8	33.8	0.31
Laetiporus gilbertsonii CA6 C2D	36.986923	-122.04234	0.33	4.14	0.12	1.47	12.5	30.5	0.33
Laetiporus huroniensis HMC1 C2H	46.824261	-87.74369	0.23	3.39	0.41	1.22	10.1	25.9	0.58
Mycocacia meridionalis FP150352 C4E	18.181083	-76.725883	0.57	1.3	0.84	1.19	12.9	25.8	0.23
Merulius tremullosus FP102301 C3E	43.086646	-89.42099	0.79	10.62	0.08	1.19	17.6	30.4	0.26
Merulius tremellosus FP150849 C3F	16.958087	-88.980764	0.84	9.62	0.02	1.24	18.2	30.3	0.28
Phlebiopsis flavidoalba FP102185 B12D	37.530421	-88.432503	0.59	8.04	0.05	1.57	16.2	30.7	0.33
Phlebiopsis flavidoalba FP150451 A8G	18.181083	-76.725883	0.99	10.8	0.04	2.54	17	27.4	0.58
Phellinus gilvus HHB11977 C4H	42.694296	-86.195827	0.46	4.04	0.03	1.4	18.6	31.5	0.6
Phellinus hartigii DMR94 44 A10E	44.931524	-87.175702	0.49	1.54	1.8	1.57	18.6	19.1	0.65
Porodisculus pendulus HHB13576 B12C	43.55015	-89.756867	0.46	4.06	0.32	1.24	15	26.4	0.64
Phellinus robiniae FP135708 A10G	43.013434	-89.737043	0.52	2.3	0.07	1.53	12.3	30.2	0.63
Phellinus robiniae AZ15 A10H Banik/Mark	34.372912	-110.62454	0.23	2.14	0.12	1.48	11.8	28.7	0.62
Phlebia acerina MR4280 B9G	35.518255	-83.325863	1	8.75	0.14	1.19	17.7	27.7	0.24
Phlebia acerina DR60 A8A	19.069338	-70.795913	0.97	8.51	0.27	1.28	18.6	26.6	0.71
Pycnoporus sanguineus PR SC 95 A11C	18.105392	-67.914763	0.7	4.97	0.02	1.71	17.9	37.8	0.57
Schizophyllum commune TJV93 5 A10A	43.227069	-89.723847	0.66	4.41	0.53	2.2	19.3	32.3	0.73
Schizophyllum commune PR1117	18.25992	-65.969653	0.59	2.57	0.59	2.32	14	32.8	0.82
Tyromyces chioneus HHB11933 B10F	46.624065	-88.47299	0.81	3.88	0.06	1.19	14.6	30.6	0.22
Xylobolus subpileatus FP102567 A11A	30.679683	-89.760665	0.49	0.77	1.74	4.96	28.5	22.2	0.88

Supplementary Table 2. Species-specific relationships with trait values and dominance-tolerance trade-offs. Each species with more than one isolate was included as an indicator variable, such that the intercept reflects the overall average for those species with a single isolate. Coefficients were estimated using standard linear regression, with two-sided p-values calculated using the standard normal approximation (n=37 biologically independent replicates for all comparisons). Numbers in parentheses gives the standard error of the effect size.

Species	Thermal optima (°C)	Moisture optima (MPa)	Thermal niche width	Moisture niche width	Competitive ranking	Combined trade-off	Thermal trade-off	Moisture trade-off
Armillaria gallica	-1.408 p = 0.361 (1.515)	-0.314 p = 0.001 (0.085)	0.082 p = 0.437 (0.104)	0.439 p = 0.0002 (0.099)	-0.252 p = 0.001 (0.068)	-0.371 p = 0.004 (0.115)	-0.303 p = 0.014 (0.114)	-0.553 p = 0.0001 (0.117)
Armillaria tabescens	-1.683 p = 0.524 (2.604)	0.025 p = 0.866 (0.146)	0.092 p = 0.609 (0.178)	0.238 p = 0.171 (0.169)	-0.293 p = 0.018 (0.116)	-0.241 p = 0.232 (0.197)	-0.236 p = 0.241 (0.196)	-0.376 p = 0.072 (0.201)
Hyphoderma setigerum	-1.083 p = 0.681 (2.604)	0.060 p = 0.684 (0.146)	-0.263 p = 0.152 (0.178)	-0.128 p = 0.458 (0.169)	0.185 p = 0.124 (0.116)	0.280 p = 0.166 (0.197)	0.450 p = 0.030 (0.196)	0.291 p = 0.158 (0.201)
Merulius tremellosus	2.517 p = 0.343 (2.604)	0.200 p = 0.182 (0.146)	0.130 p = 0.472 (0.178)	-0.182 p = 0.291 (0.169)	0.337 p = 0.008 (0.116)	0.494 p = 0.019 (0.197)	0.312 p = 0.124 (0.196)	0.526 p = 0.015 (0.201)
Phlebiopsis flavidoalba	1.217 p = 0.645 (2.604)	0.015 p = 0.919 (0.146)	0.077 p = 0.668 (0.178)	0.218 p = 0.210 (0.169)	0.313 p = 0.012 (0.116)	0.407 p = 0.049 (0.197)	0.380 p = 0.064 (0.196)	0.303 p = 0.143 (0.201)
Phellinus robiniae	1.617 p = 0.540 (2.604)	-0.155 p = 0.297 (0.146)	-0.161 p = 0.374 (0.178)	0.049 p = 0.776 (0.169)	-0.100 p = 0.397 (0.116)	-0.015 p = 0.940 (0.197)	0.117 p = 0.557 (0.196)	-0.051 p = 0.802 (0.201)
Phlebia rufa	-0.683 p = 0.795 (2.604)	-0.005 p = 0.973 (0.146)	0.140 p = 0.440 (0.178)	-0.163 p = 0.346 (0.169)	0.510 p = 0.0002 (0.116)	0.802 p = 0.0004 (0.197)	0.610 p = 0.005 (0.196)	0.832 p = 0.0003 (0.201)
Schizophyllum commune	4.717 p = 0.081 (2.604)	-0.305 p = 0.046 (0.146)	0.069 p = 0.701 (0.178)	0.293 p = 0.096 (0.169)	0.149 p = 0.212 (0.116)	0.120 p = 0.547 (0.197)	0.107 p = 0.591 (0.196)	-0.021 p = 0.916 (0.201)
Intercept (species avg.)	27.833 p = 0.000 (0.893)	-0.470 p = 0.000 (0.050)	0.539 p = 0.000 (0.061)	0.240 p = 0.0003 (0.058)	0.476 p = 0.000 (0.040)	0.165 p = 0.022 (0.068)	-0.098 p = 0.158 (0.067)	0.129 p = 0.071 (0.069)
Observations	37	37	37	37	37	37	37	37
R ²	0.212	0.459	0.177	0.531	0.724	0.646	0.567	0.714
Adjusted R ²	-0.013	0.305	-0.058	0.397	0.645	0.544	0.444	0.632
F (df = 8; 28)	0.940	2.974*	0.751	3.967**	9.169***	6.375***	4.588**	8.739***

Supplementary Table 3. The 19 WorldClim climate variables and their correlations with the three climate PCA variables used in the regression analysis. Shown only are those correlations which are significant at the $p=0.05$ level after adjusting for multiple comparisons via the Hochberg method. $N=37$ biologically independent replicates were used for the climate principal component analysis.

	PCA 1	PCA 2	PCA 3
Annual Mean Temperature	–	–	–
Mean Diurnal Range	–	–	0.49 ($p = 0.002$)
Isothermality	–	-0.44 ($p = 0.007$)	–
Temperature Seasonality	–	0.44 ($p = 0.007$)	0.34 ($p = 0.039$)
Max Temperature of the Warmest Month	–	–	0.84 ($p = 0.002$)
Min Temperature of the Coldest Month	–	–	–
Temperature Annual Range	–	0.43 ($p = 0.009$)	0.38 ($p = 0.019$)
Mean Temperature of the Wettest Quarter	0.63 ($p = 3e^{-5}$)	–	0.59 ($p = 1e^{-4}$)
Mean Temperature of the Driest Quarter	–	–	–
Mean Temperature of the Warmest Quarter	–	–	0.64 ($p = 2e^{-5}$)
Mean Temperature of the Coldest Quarter	–	–	–
Annual Precipitation	–	–	–
Precipitation of the Wettest Month	–	-0.47 ($p = 0.004$)	-0.38 ($p = 0.019$)
Precipitation of the Driest Month	–	–	–
Precipitation Seasonality	–	-0.95 ($p = 1e^{-12}$)	-0.39 ($p = 0.016$)
Precipitation of the Wettest Quarter	–	-0.40 ($p = 0.014$)	-0.37 ($p = 0.025$)
Precipitation of the Driest Quarter	–	–	–
Precipitation of the Warmest Quarter	0.39 ($p = 0.017$)	–	–
Precipitation of the Coldest Quarter	-0.66 ($p = 8e^{-6}$)	–	-0.34 ($p = 0.035$)

Supplementary Table 4. The relationship between climate and performance traits.

Climate variables had negligible ability to predict competitive ranking or niche width individually, with no variables significant and with low R^2 . Coefficients were estimated using standard linear regression, with two-sided p-values calculated using the standard normal approximation, and adjusted for multiple comparisons using the Benjamini-Hochberg method. Numbers in brackets denote the 95% confidence interval for the effect size, and numbers in parentheses denote the corresponding phylogenetic least squares coefficients (n=37 biologically independent replicates for all comparisons).

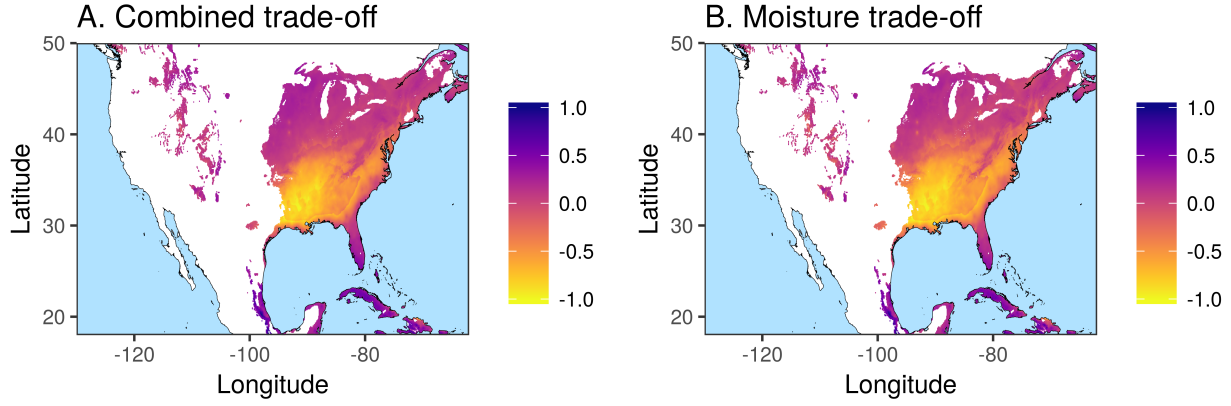
	Competitive ability	Thermal niche width	Moisture niche width
Climate PCA 1	0.073 (0.060) [-0.00, 0.15] p = 0.15	-0.093 (-0.090) [-0.19, 0.01] p = 0.16	-0.038 (-0.020) [-0.15, 0.07] p = 0.63
Climate PCA 2	0.016 (0.004) [-0.04, 0.07] p = 0.66	0.061 (0.055) [-0.01, 0.13] p = 0.17	0.008 (-0.010) [-0.07, 0.08] p = 0.83
Climate PCA 3	-0.020 (-0.011) [-0.07, 0.03] p = 0.63	0.016 (0.012) [-0.05, 0.08] p = 0.67	0.054 (0.039) [-0.02, 0.13] p = 0.26
R^2_{adj}	0.08	0.12	0.12
F (df= 3; 32)	1.37 ($p = 0.28$)	2.84 ($p = 0.05$)	1.08 ($p = 0.37$)
AIC	-22.30	-2.22	4.36
Pagel's λ	0.64	0.11	0.55
L.R.T. $\lambda = 0$	$\chi^2 = 15.9^{***}$ (0.0001)	$\chi^2 = 0.30$ ($p = 0.58$)	$\chi^2 = 7.0^*$ ($p = 0.008$)
AIC (PGLS)	-8.2	-3.8	5.9

Supplementary Table 5. The corresponding regression results using linear mixed models (LMMs), with species as a random effect rather than a fixed effect. The coefficient estimates are virtually unchanged relative to the fixed effect models (Table 2, main text). Because of the inability to compute an exact R^2 when using LMMs, we report the results of the fixed effects models in the main text. Unadjusted two-sided p-values were calculated using Satterthwaite's approximations, using the lmerTest package in R (n=37 biologically independent replicates for all comparisons). Numbers in parentheses denote standard errors.

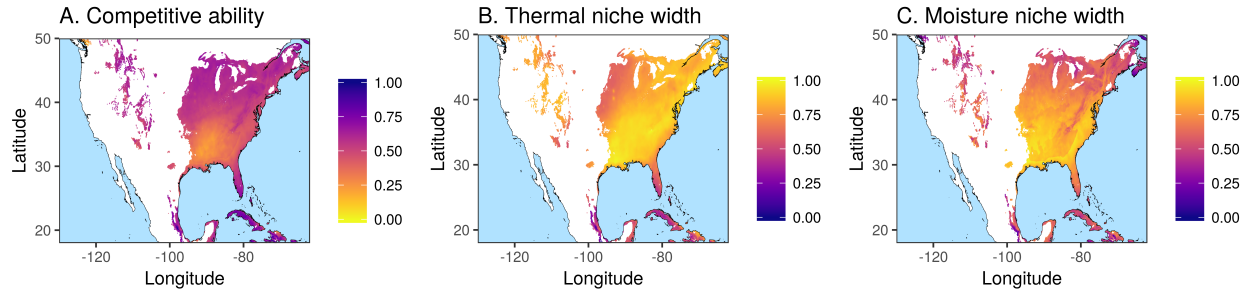
	Thermal \times moisture dominance-tolerance	Thermal dominance-tolerance	Moisture dominance-tolerance	Competitive ability	Thermal niche width	Moisture niche width
Climate PCA 1	0.196 p = 0.0002 (0.051)	0.196 p = 0.0001 (0.050)	0.148 p = 0.011 (0.058)	0.077 p = 0.038 (0.037)	-0.092 p = 0.054 (0.047)	-0.047 p = 0.385 (0.054)
Climate PCA 2	-0.026 p = 0.474 (0.036)	-0.083 p = 0.020 (0.035)	-0.014 p = 0.736 (0.041)	0.014 p = 0.590 (0.026)	0.061 p = 0.070 (0.034)	0.006 p = 0.869 (0.038)
Climate PCA 3	-0.084 p = 0.014 (0.034)	-0.035 p = 0.298 (0.034)	-0.075 p = 0.059 (0.040)	-0.024 p = 0.349 (0.025)	0.014 p = 0.636 (0.030)	0.062 p = 0.085 (0.036)
L.R.T	$\chi^2=15.5^{**}$	$\chi^2=19.5^{***}$	$\chi^2=8.5^*$	$\chi^2=4.6$	$\chi^2=9.4^*$	$\chi^2=4.2$
AIC	33.6	27.8	40.1	10.8	17.7	28.5

Supplementary Table 6. Univariate tests of phylogenetic signal for climate PCA variables and performance traits. Estimates were obtained using the `phylosig` function in the `phytools` package in R, and two-sided p-values were adjusted for multiple comparisons using the Benjamini-Hochberg method. Only moisture niche width and competitive ranking showed significant signal, such that the combined moisture temperature dominance tolerance trade-off and moisture dominance tolerance trade-off likewise showed significant phylogenetic conservatism. Conversely, climate variable and thermal niche variables exhibited negligible phylogenetic signal. These relationships mirror the results of phylogenetic least squares regression (Table 2, main text).

	Pagel's λ	<i>P</i> -value
Climate		
Precipitation PC 1	<0.01	1.00000
Precipitation PC 2	0.15	1.00000
Precipitation PC 3	<0.01	1.00000
Precipitation PC 4	0.09	1.00000
Precipitation PC 5	0.47	0.21547
Temperature PC 1	<0.01	1.00000
Temperature PC 2	<0.01	0.24408
Temperature PC 3	<0.01	1.00000
Temperature PC 4	<0.01	1.00000
Temperature PC 5	<0.01	1.00000
Performance traits		
Dominance-tolerance (combined)	0.71	0.00311
Dominance-tolerance (temperature)	0.39	0.09919
Dominance-tolerance (moisture)	0.70	0.00002
Competitive ranking	0.65	0.00013
Temperature niche width	0.14	1.00000
Moisture niche width	0.58	0.00168
Optimal moisture	0.26	0.54829
Optimal Temperature	0.27	1.00000



Supplementary Figure 1. The combined thermal \times moisture dominance tolerance trade-off and the moisture trade-off, as predicted by the regression results (Table 2). These two trade-offs show similar relationships to the thermal trade-off, albeit with lower explanatory power. The results are obtained by applying the linear regression results ($n=37$ biologically independent isolates) to the climate data for each pixel. High values (~ 1.0) indicate high competitive ability and low stress tolerance; low values (-1) indicate high stress tolerance and low competitive ability.



Supplementary Figure 2. The relationships between climate and competitive ability, thermal niche width, and moisture niche width. The results are obtained by applying the linear regression results ($n=37$ biologically independent isolates) to the climate data for each pixel. High values (~ 1.0) indicate high competitive ability in (a) and wide niche widths in (b) and (c).