COMPARATIVE PLASTICITY OF RARE *PITYOPSIS RUTHII* AND ITS COMMON CONGENER *P. GRAMINIFOLIA*

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ABSTRACT

The natural world is dynamic, and species must successfully respond to the environmental changes they experience. *Pityopsis ruthii* (Ruth's golden aster) is an imperiled endemic perennial confined to boulder crevices along short stretches of the Hiwassee and Ocoee Rivers in Polk County, TN. To investigate plasticity of this species within the context of such change, we used a comparative approach to observe general differences in plasticity between *P. ruthii* and its widespread congener *P. graminifolia* by assessing the responses of individuals to experimentally imposed environmental change. Overall, *P. ruthii* exhibited lower plasticity than *P. graminifolia*, but these differences were resource-dependent with *P. ruthii* having significantly greater plasticity within the context of water availability while *P. graminifolia* exhibited greater plasticity within the context of light availability and temperature. The plasticity differences of *P. ruthii* and its positive associations with productivity provide context for understanding its constrained distribution and habitat specificity.

DEDICATION

To my wife, Holly, who has always supported me and encouraged me to be not only a great scientist but a better person. You are the best person I know and the one I strive to do better for. I love you so much. And to our future little scientist whom I hope, with our help, will change the world.

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CHAPTER I

INTRODUCTION

The question of why some species are rare while others are common is long-standing within the biological community (Darwin 1859, Stebbins 1942, Kruckeberg & Rabinowitz 1985, May 1999, Murray et al. 2002, McGill 2006, Wiegand et al. 2020). Understanding the factors influential to species' rarity and the responses of those factors to environmental change has important implications for ecological theory as it relates to differences in species' relative abundance, as well as to the conservation of species and overall biodiversity (Bevill & Louda 1999). While invasive plant species, at the extreme opposite end of the spectrum of relative abundance, have been widely researched (see reviews by Daehler 2003, Cadotte et al. 2006, Pyŝek & Richardson 2007, van Kleunen & Fischer 2009, Vanderhoven et al. 2010, van Kleunen et al. 2010, Davidson et al. 2011, Palacio-López & Gianoli 2011, Leffler et al. 2014), research on rare plant species has been relatively limited (but see reviews by Murray et al. 2002, Farnsworth 2006, Combs et al. 2013). It has been suggested that knowledge of the biological characteristics of invasive species as elucidated from a richer body of reported research could be directly applicable to understanding species rarity as an opposite condition, but research of this concept has produced mixed results (see Bradshaw et al. 2008, Jeschke & Strayer 2008, Blackburn & Jeschke 2009, Pandit et al. 2011, Schmidt et al. 2012), suggesting that rare species merit distinct research attention.

Pityopsis ruthii (Small) Small (Asteraceae; Ruth's golden aster) is a rare plant species restricted primarily to soil-filled cracks in boulders on exposed banks along 5.7 km of the Hiwassee River and 4.6 km of the Ocoee River in Polk County, Tennessee, USA downstream of dams operated by the Tennessee Valley Authority (TVA). In total, the TVA has delineated 67 distinct occurrences or clusters of this species with individual occurrences ranging in size from <5 to ~1000 plants (personal communication, A. Dattilo, Botanist, TVA). Endemism is the most common type of species rarity and the type that has traditionally received the most attention from conservationists (Rabinowitz 1981, May 1988), and we categorize *P. ruthii* as 'endemic' based on its small geographic range, narrow habitat specificity, and large size of at least a single occurrence (see Rabinowitz 1981). Given its rarity and associated conservation concerns, *P. ruthii* is listed as both federally and State endangered (ESA; USFWS 1985) and is ranked G1 (critically imperiled) by NatureServe (2009). Although *P. ruthii* is notably rare, other species of *Pityopsis* differ dramatically in their relative abundance. The most abundant species in the genus, *P. graminifolia* (Michx.) Nutt. (narrowleaf silkgrass), occurs throughout the southeastern United States and northern Central America across a variety of habitat types (Semple 2006).

Research comparing rare plant species with closely related common plant species has the potential to advance ecological theory about species rarity by revealing consistent patterns while providing control of the potential influences of life history and phylogeny on comparative outcomes (Kunin & Gaston 1997, Godt & Hamrick 2001, Murray et al. 2002, Farnsworth 2006). Comparisons of the genetic diversity of rare and common congeners at both population and species levels have suggested that rare species tend to have lower levels of genetic diversity than their widespread plant congeners (see reviews by Gitzendanner & Soltis 2000, Cole 2003), which could impede their ability to adapt to environmental change. However, plasticity could allow for acclimation to environmental change without any associated genetic change. Recent research that combined investigation of developmental plasticity in response to seasonal temperature change with measures of genetic diversity of rare and common species within a single genus revealed that plasticity in growth responses could help to explain differences in geographical distribution when genetic diversity did not (Lovell & McKay 2015).

Although a range of qualitative and quantitative life history characteristics and ecological traits related to growth, reproduction, and abiotic and biotic interactions also have been compared between rare and common congeneric plant species, attempted syntheses of this body of research have concluded that most foci were the subject of single-to-few studies and studies with shared foci often generated mixed findings (Bevill & Louda 1999, Murray et al. 2002). Some particularly comprehensive but location-specific studies comparing life history traits within suites of primarily congeneric pairs of rare and common species (as defined by geographic distribution) have suggested that rare endemic species generally may be shorter in stature, produce fewer but larger seeds, and less likely to reproduce vegetatively than are common species (Lavergne et al. 2004, Farnsworth 2006). Among studies that have used a trait-based approach to compare rare and common plant species, foci on the mean values of traits associated with growth, reproduction, and abiotic and biotic interactions have been common (Murray et al. 2002). In comparison, relatively few studies have included comparisons of ecophysiological traits that could underlie more observable traits (e.g., Baskauf & Eickmeier 1994, Schulze et al. 1996, Baskin et al. 1997, Osunkoya & Swanborough 2001, Cleavitt 2002, Lavergne et al. 2004, Pohlman et al. 2005, Dangremond et al. 2015). Similarly, investigations of plasticity in this context have remained limited (e.g., Pohlman et al. 2005, Denton et al. 2007, Runk & Zobel 2007, Marchin et al. 2009, Lovell & McKay 2015) although plasticity of key traits also could influence organismal fitness and species performance (Nicotra & Davidson 2010, Godoy et al. 2012). In studies of invasive species, researchers have reported positive associations between invasiveness – as a form of extreme commonness – and plasticity (Ruprecht et al. 2014), particularly when ecophysiological traits were considered (Funk 2008, Davidson et al. 2011, Godoy et al. 2012), demonstrating the potentially important role that ecophysiological traits and plasticity of such traits could play in species rarity and commonness.

The rapid pace of contemporary environmental change due to anthropogenic activities and influences (Palumbi 2001) has been implicated as a particular threat to rare species (Mouillot et al. 2013), and a detailed comparison of plasticity could provide insight into the relative ability of rare species to acclimate to such change. For the narrow endemic, *P. ruthii*, the active management of the rivers along which this species is found profoundly influences its habitat and changes in this management regime would be associated with environmental changes that could impact the species. Currently, the habitat of *P. ruthii* is characterized by moisture availability that range widely from frequent, often prolonged drought-like conditions to periodic high-flow events that produce total inundation (Moore et al. 2016). Population monitoring data collected for this species following several high-flow dam releases indicates that longer term inundation may negatively impact occurrences (unpublished report, TVA). In contrast, reduced water flow regimes could allow for the encroachment of other plant species along the riverbanks, and it has been suggested that *P. ruthii* is sensitive to shading by surrounding vegetation (Thomson & Schwartz 2006). In addition to environmental changes associated with river management, *P. ruthii* will experience increasing temperatures associated with global climatic warming, and influence of temperature on this species are unknown (Moore et al. 2016). Toward elucidating the potential responses of *P. ruthii* to changes in light, moisture, and temperature, we investigated the plasticity of individuals propagated from naturally occurring field populations of each species as a potential acclimatory constraint.

Methodology

Seed collection & propagation

We aimed to obtain seed from throughout the ranges of both rare *P. ruthii* and common *P. graminifolia* so as to account for potential genetic and phenotypic variation across locations. Seeds of rare *P. ruthii* were collected in October 2017 from two occurrences along the Hiwassee River (H1 and H2) and two occurrences along the Ocoee River (O1 and O1; Polk County, Tennessee, USA); selected occurrences were separated by at least 2 km such that gene flow between occurrences was unlikely. Seed of widespread *P. graminifolia* were collected in October and November 2017 from five locations: Ocoee River (TN; Polk County, Tennessee), Black Mountain Road (GA1; Stephens County, Georgia, USA), Currahee Mountain (GA2; Stephens County, Georgia), Little Manatee River State Park (FL; Hillsborough County, Florida, USA), and Zube Park (TX; Harris County, Texas, USA; Table 1). All *P. ruthii* seeds and *P. graminifolia* seeds from the Tennessee and Georgia occurrences were collected by the author; *P. graminifolia* seeds from the Florida and Texas occurrences were collected by local contacts made through the biodiversity information platform *iNaturalist* (http://inaturalist.org). For both species, numerous seeds from each of 15-25 distinct parent individuals per occurrence were sampled. Collected seeds were deposited in paper bags and stratified in cold storage for a period of four months prior to germination.

Table 1 Locations of natural occurrences of *Pityopsis ruthii* and *P. graminifolia* from which seeds were collected to propagate offspring for plasticity experiments and genetic investigations

Species	Site	County, State	Latitude	Longitude	Elevation (m)
P. ruthii	Hiwassee River 1 (H1)	Polk Co., TN	35.17593	-84.39513	317
	Hiwassee River 2 (H2)	Polk Co., TN	35.18130	-84.40772	271
	Ocoee River 1 (O1)	Polk Co., TN	35.08117	-84.52805	277
	Ocoee River 2 (O2)	Polk Co., TN	35.09188	-84.53164	262
P. graminifolia	Ocoee River (TN)	Polk Co., TN	35.09188	-84.53164	262
	Black Mountain Road (GA1)	Stephens Co., GA	34.64799	-82.88583	208
	Curahee Mountain (GA2)	Stephens Co., GA	34.52865	-83.37592	502
	Little Manatee River (FL)	Hillsborough Co., FL	27.67007	-82.40177	4
	Zube Park (TX)	Harris Co., TX	30.02872	-95.81427	62

Stratified seeds were sorted by hand to identify rounded, filled seeds (containing a mature embryo) for planting as detailed for *P. ruthii* by Wadl et al. (2014). Approximately 6-8 filled seeds from each parent individual from each occurrence were sowed into each of four 7-cm^2 × 8.5-cm-deep pots filled with a commercially available potting medium (Pro-Mix Bx Biofungicide + Mycorrhizae, Premier Tech Horticulture, Quakertown, Pennsylvania, USA). The four pots containing seeds from each parent plant were then randomly assigned to each of four controlled-environment growth chambers (model PGR15, Conviron Controlled Environments Limited, Winnipeg, Manitoba, CAN) such that each chamber contained one pot of seed from each parent plant. All chambers were set initially to provide a 12-h photoperiod at a constant 25°C. All pots were watered similarly as needed to keep the soil moist during a 1-month germination period. Germination was highly successful and following the germination period, each pot was thinned to the single individual that exhibited the earliest third leaf development. To minimize the chance of growing plants becoming root bound during the course of the experiments, these individuals and the surrounding soil were then carefully transplanted into separate 11-cm^2 × 9.5-cm-deep pots filled with the same commercially available potting medium.

Environmental treatments

To assess plasticity of *P. ruthii* and *P. graminifolia* in response to light, temperature, and soil moisture, we conducted three simultaneous experiments with the four controlled-environment growth chambers. Following the germination period, one chamber (i.e., the ‘ambient’ chamber) was programmed to provide temperature and light conditions to replicate field conditions during the *P. ruthii* growing season based on historical weather data (NOAA 2018) and field measurements, respectively. This chamber was programmed to provide temperature ranging night-day from 20-30°C based on three decades of weather data from an observation station closest to the location where *P. ruthii* is found (NOAA 2018) and a 12-h photoperiod with a maximum daily light level of 600 umol photons m^{-2} s^{-1} in accordance with our field measurements in *P. ruthii* habitat. Soil moisture availability in *P. ruthii* is highly variable across time due to a combination of precipitation and river management and flow regimes; pots in the ambient chamber were watered as needed to 50% field capacity, determined by weighing a subset of pots of each species every 2 days in accordance with the methods of Liu et al. (2005).

The three other growth chambers were programmed to provide the same conditions as the ambient chamber but each with a contrasting level of a single environmental condition (light, temperature, or soil moisture) to mimic how that abiotic factor could change as a result of significant threats to *P. ruthii* according the U.S. Fish and Wildlife Service (USFWS 2007; personal communication, G. Call, Recovery Specialist, USFWS). Specifically, the chamber set to provide altered light availability (i.e., the 'light' chamber) was programmed to provide the same conditions as the ambient chamber but with a maximum daily light level of 300 photons umol m^{-2} s^{-1} (i.e., a 50% reduction) based on the measured PAR of forest habitat along the edges of *P. ruthii* habitat with ~50% canopy cover. The chamber set to provide altered temperature (i.e., the 'temperature' chamber) was programmed to provide the same conditions as the ambient chamber but with temperature ranging night-day from 22-32°C to simulate average projections of global temperature increase for this century (IPCC 2014). The chamber subject to altered soil moisture availability (i.e., the 'water' chamber) was programmed to provide the same conditions as the ambient chamber but pots in this chamber were watered to 100% field capacity to represent increased inundation along the currently dammed rivers where *P. ruthii* is found. The positions of pots within each chamber were rotated weekly to control for spatial differences in microclimate and treatment levels assigned to each chamber were reassigned monthly with all plants moved accordingly to minimize any chamber effects and alleviate issues of pseudoreplication (Gibson 2014).

Data collection

All individuals were grown for 6 months in the growth chambers following treatment initiation during which growth, phenological, and ecophysiological data were collected. Growth was assessed biweekly by counting the numbers of leaves, stems, and buds/flowers and measuring the shoot height of each individual. Maximum values of growth measures were determined from these repeated measurements; maximum values were used to calculate leaf production and shoot elongation rates. To investigate potential physiological processes underlying observable performance, instantaneous rates of leaf-level photosynthesis were measured at 3 months after treatment initiation using a portable gas-exchange system (6800XT, LI-COR, Inc., Lincoln, Nebraska, USA) for the single youngest fully expanded leaf for all individuals with leaves large enough to fill the cuvette. All photosynthesis measurements were made within 2 h of the middle of the daily photoperiod during a 3-week measurement period.

Measurements were made after gas exchange had equilibrated as was determined when the coefficient of variation for the CO_2 partial pressure differential between the sample and reference analyzers was below 1% with an average wait time of 3-5 minutes. Conditions of PAR and temperature inside the cuvette were set to match the environmental conditions inside the growth chamber occupied by each measured individual. For all measurements, a constant external CO_2 partial pressure of 40 Pa was provided by a CO_2-control module and water vapor pressure deficit of the sample air was maintained between 1.0 and 1.5 kPa. The range of CO_2 depletion in the chamber that defined the CO_2 concentration around the leaf was generally <1 Pa.

At 6 months after treatment initiation, all individuals were destructively harvested to assess productivity, biomass allocation, and leaf structure. Harvests involved removing each individual from its pot and thoroughly washing the soil away from roots. Root length was measured as the distance from the start of the green shoot to the tip of the longest root when plants were held upright. A single young fully expanded leaf from each individual was removed and fully dried in a laboratory oven to determine specific leaf area. The remaining whole plants were sorted into leaves, stems (for *P. graminifolia* only as *P. ruthii* lacks discernable stems), roots, and buds/flowers and also dried to determine dry mass of each component for each individual. These values were summed to yield total biomass per individual. To investigate biomass allocation, we calculated root-shoot ratio, shoot mass fraction, root mass fraction, root length ratio, and specific root length. Units, calculations, and other details for all measured and calculated traits are provided in Table 1.

Plasticity determination

A relative distances plasticity index (RDPI; see Valladares et al. 2006) was used to calculate trait plasticity within the context of light, temperature, and soil moisture differences for species and occurrences within species for all assessed growth, allocation, and leaf traits. The RDPI is based on the absolute phenotypic distances of genotypes across different environments and allows for statistical comparison of plasticity for species and populations within species (i.e., occurrences; Valladares et al. 2006). We used the index to calculate individual-level trait plasticity across siblings within the context of light, temperature, and soil moisture as:

$$\text{RDPI} = d_{ij \to i'j'}/(x_{i'j'} + x_{ij})$$

where j and j' are two individuals of the same species or occurrence (we compared offspring of the same parent), i and i' represent two different environments (i.e., ambient vs. reduced light, ambient vs. elevated temperature, ambient vs. increased water in our experiment), $d_{ij \to i'j'}$ is the distance among trait values for the pair of individuals (with distance defined as the absolute value of the difference in trait values), and $x_{i'j'} + x_{ij}$ is the sum of the trait values (see Valladares et al. 2006). RDPI values range from 0 (no plasticity) to 1 (maximum plasticity); this standardized range can allow for comparisons across traits.

Statistical analyses

We determined if the proportions of plants that survived and produced flowers differed between *P. ruthii* and *P. graminifolia* with Fisher's exact tests and between occurrences within species with likelihood ratio tests (LRT). In the event of a significant main effect of occurrences, comparisons among occurrences were made with subsequent pairwise comparisons. To investigate potential differences in the responses of *P. ruthii* and *P. graminifolia* to light, temperature, and water availability, we used two-way multivariate analyses of variance (MANOVA) to evaluate the interactions of species and each environmental treatment (i.e., light, temperature, water) on the grouped growth and allocation dependent variables listed in Table 2. We similarly used a two-way analysis of variance (ANOVA) to investigate the interactions of species and each environmental treatment on leaf structure, physiology, and total biomass as individual dependent variables (Table 2). To directly investigate potential differences in trait plasticity of *P. ruthii* and *P. graminifolia* within the context of modified light, temperature, and water conditions, we used one-way MANOVA with grouped traits or one-way ANOVA with individual traits to evaluate the main effects of species (and occurrence within species) on trait plasticity values. Correlation between dependent variables included in the MANOVA tests was verified with Pearson correlations.

Table 2 Measured traits of *Pityopsis ruthii* and *P. graminifolia* in plasticity experiments

Trait	**Description**	**Measurement**
Growth		
Height	Shoot height	cm
Leaves	Number of leaves	number
Stems	Number of stems	number
LPR	Leaf production rate	number day^{-1}
SER	Shoot elongation rate	mm day^{-1}
RL	Root length	cm
Allocation		
RSR	Root-shoot ratio	root g^{1} shoot g^{-1}
SMF	Stem mass fraction	stem g total biomass g^{-1}
RMF	Root mass fraction	root g total biomass g^{-1}
SRL	Specific root length	root cm root g^{1}
RLR	Root length ratio	root cm total biomass ^{1}
Leaf Morphology		
SLA	Specific leaf area	cm g^{-1}
Leaf Physiology		
A	Photosynthetic rate	µmol m^{-2} s^{-1}
Productivity		
Biomass	Total dry biomass	g

A significant species-level MANOVA was followed by analysis of variance (ANOVA) to assess the main effects and interactions of species (or occurrence) and the associated environmental treatments on the included individual dependent variables. Plasticity values for offspring of the same parent were regressed against the mean total biomass (as a fitness proxy) of those offspring across relevant environmental treatment levels to ascertain whether plasticity was adaptive (i.e., positively correlated with fitness), maladaptive (i.e., negative correlated with fitness), or neutral (i.e., not correlated with fitness). Results of statistical tests were considered significant if $p \leq 0.05$. All statistical analyses were performed in SPSS (SPSS Statistics Version 26 software, IBM Corp., Armonk, New York, USA).

Results

Germination, survival, & flowering

Germination was highly successful for all occurrences except for the FL *P. graminifolia* occurrence. Nearly all pots planted with seeds from all other occurrences produced at least one successful germinant. Across all environmental conditions, survival also was highly successful for both species. In total, 99.3% of *P. ruthii* individuals and 97.8% of *P. graminifolia* individuals used in our plasticity experiments survived to harvest (Table 3). All individuals of both species survived in the ambient conditions and when water was increased, while differences in survival between species in reduced light and elevated temperature were not significant ($p = 0.309$ and 0.358, respectively). Observable differences in the survival of *P. ruthii* from different occurrences when grown in elevated temperature were not significant (LRT = 3.405, $df = 3$, $p = 0.333$). Similarly, observable differences in the survival of *P. graminifolia* occurrences when light was reduced and temperature was elevated were not significant (LRT = 2.240, *df* = 3, *p* = 0.520 and LRT = 3.394, *df* = 3, *p* = 0.335, respectively).

Table 3 Number of individuals propagated from field-collected seed from occurrences of rare *Pityopsis ruthii* and common *P. graminifolia* that survived and flowered within environmental treatments (ambient, modified light, modified temperature, modified water) in plasticity experiments

Treatment	**Species**	**Occurrence**	***n***	**survived**	**flowered**
Ambient	*P. ruthii*	H1	19	19	0
		H2	22	22	2
		O1	15	15	0
		O2	19	19	0
	P. graminifolia	TN	17	17	0
		GA1	13	13	1
		GA2	9	9	4
		TX	9	9	2
Light	*P. ruthii*	H1	19	19	1
		H2	22	22	9
		O1	15	15	3
		O2	19	19	0
	P. graminifolia	TN	16	15	3
		GA1	13	13	2
		GA2	9	9	1
		TX	9	9	0
Temperature	*P. ruthii*	H1	18	17	1
		H2	22	22	0
		O1	14	13	0
		O2	19	19	0
	P. graminifolia	TN	14	12	1
		GA1	12	11	3
		GA2	9	9	7
		TX	8	8	2
Water	*P. ruthii*	H1	19	19	0
		H2	22	22	0
		O1	15	15	0
		O2	19	19	0
	P. graminifolia	TN	17	17	3
		GA1	13	13	7
		GA2	9	9	1
		TX	9	9	2

Overall, <25% of plants produced flowers during the plasticity experiments, but flowering was 4-fold greater in *P. graminifolia* than in *P. ruthii* ($p < 0.001$; Table 3). Flowering in *P. graminifolia* also was significantly greater than in *P. ruthii* in ambient conditions ($p = 0.027$) and with elevated temperature ($p < 0.001$) and water ($p < 0.001$), although flowering did not differ between species when light was reduced ($p = 0.804$). Flowering did not differ among occurrences of *P. ruthii* in ambient conditions (LRT = 5.040, $df = 3$, $p = 0.539$) and in elevated temperature (LRT = 2.843, $df = 3$, $p = 0.416$), but flowering did differ among *P. ruthii* occurrences when light was reduced (LRT = 16.555, $df = 3$, $p < 0.001$). Specifically, in low light, flowering of plants from the H2 occurrence was greater than for occurrences H1 and O2 ($p = 0.011$ and $p = 0.002$, respectively). When water was increased relative to ambient conditions, *P. ruthii* did not flower. Flowering differed among occurrences of *P. graminifolia* in ambient conditions (LRT = 10.929, $df = 3$, $p = 0.013$) and with increased water availability (LRT = 10.826, $df = 3$, $p = 0.013$). Specifically, flowering was greater in occurrence GA2 than TN in ambient conditions ($p = 0.008$) and greater in GA1 than GA2 with more water ($p = 0.017$). Flowering among occurrences of *P. graminifolia* did not differ when light was reduced (LRT = 0.494, $df = 3$, $p = 0.920$) or temperature was elevated (LRT = 2.085, $df = 3$, $p = 0.416$) relative to ambient conditions.

Effects of environmental conditions on trait values

The interaction of species × light on growth and *A* (photosynthetic rate) of *P. ruthii* and *P. graminifolia* was significant (Tables 4,5). However, of individual growth traits, only the mean number of stems was significantly influenced by this interaction ($F_{1,244} = 6.179$, $p = 0.014$). Specifically, reduced light availability was associated with a significant decrease in both *A* and stem number in *P. ruthii*, but reduced light availability did not significantly influence these variables in *P. graminifolia* (Figure 1).

Table 4 Results of two-way multivariate analysis of variance (MANOVA) to test for the interactions of species and light, temperature, and water on grouped growth and allocation traits and results of two-way analysis of variance (ANOVA) to test for the interactions of species and light, temperature, and water on specific leaf area (SLA) and instantaneous leaf-level photosynthetic rate (*A*) of rare *Pityopsis ruthii* and common *P. graminifolia* individuals in plasticity experiments. Individual traits included in growth and allocation groups are listed in Table 2. Asterisks denote *p*-values significant at ≤ 0.05 significance level

Dependent variables	**Source**	***df***	**Wilk's lambda**	***F***	***p***
Growth	Species × light	6	0.941	2.878	0.010*
	Species × temperature	6	0.842	7.779	<0.001*
	Species × water	6	0.918	3.4867	0.003*
Allocation	Species × light	5	0.980	1.236	0.296
	Species × temperature	5	0.971	1.435	0.224
	Species × water	5	0.916	4.448	0.002*

Dependent variable	**Treatment**	***df***	**Mean square**	***F***	***p***
SLA	Species × light	1	1710.243	0.574	0.449
	Species × temperature	1	53.963	0.019	0.891
	Species × water	1	171.057	0.178	0.673
A	Species × light	1	101.307	30.072	<0.001*
	Species × temperature	1	0.208	0.029	0.866
	Species × water	1	43.842	7.740	0.006*
Biomass	Species × light	1	5.042	1.512	0.220
	Species × temperature	1	23.358	5.441	0.021*
	Species × water	1	31.810	6.627	0.011*

Table 5 Results of one-way multivariate analysis of variance (MANOVA) to test for the main effect of species on plasticity measured as a relative distances plasticity index (RDPI) on grouped growth and allocation traits and results of one-way analysis of variance (ANOVA) to test for the main effect of species on plasticity of specific leaf area (SLA) and instantaneous leaf-level photosynthesis rate (A) of rare *Pityopsis ruthii* and common *P. graminifolia* individuals. Individual traits included in growth and allocation groups and units of measure are listed in Table 2. Asterisks denote p-values significant at ≤ 0.05 significance level

Dependent variables	**Environmental condition**	***df***	**Wilk's lambda**	***F***	***p***
$\mathrm{RDPI}_{\mathrm{growth}}$	Light	6	0.879	2.515	0.026^{*}
	Temperature	6	0.829	3.646	0.002^{*}
	Water	6	0.894	2.230	0.045^{*}
$\mathrm{RDPI}_{\mathrm{allocation}}$	Light	5	0.846	2.902	0.019^{*}
	Temperature	5	0.759	5.583	$<0.001^{*}$
	Water	5	0.727	7.061	$<0.001^{*}$

Dependent variable	**Environmental condition**	***df***	**Mean square**	***F***	***p***
$\mathrm{RDPI}_{\mathrm{SLA}}$	Light	1	<0.001	0.001	0.978
	Temperature	1	0.008	0.434	0.512
	Water	1	0.012	0.486	0.488
RDPI_{A}	Light	1	0.012	0.430	0.514
	Temperature	1	0.404	12.383	0.001^{*}
	Water	1	0.150	3.887	0.053

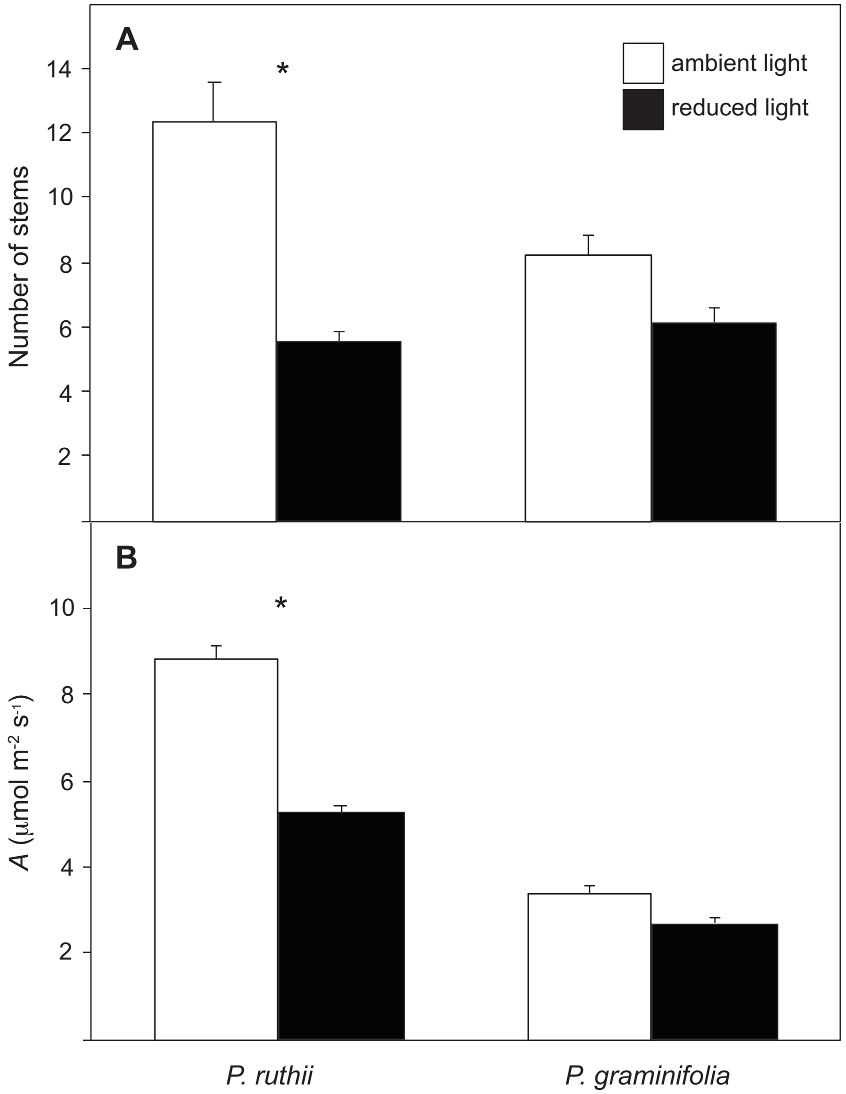

Figure 1 Mean number of stems (**A**) and instantaneous leaf-level photosynthesis rate (**B**) of individuals of rare *Pityopsis ruthii* and common *P. graminifolia* grown from field-collected seed in ambient light (600 mmol photons m^{-2} s^{-1}) and with reduced light (300 mmol photons m^{-2} s^{-1}). Error bars represent 1 SE of the mean. Asterisks denote significant differences between mean values in ambient and reduced light within species at the $p \leq 0.05$ significance level

The interaction of species × temperature on growth and total biomass of *P. ruthii* and *P. graminifolia* was significant (Tables 4 and 5). The majority of individual growth traits were significantly influenced by this interaction, including height ($F_{1,238} = 15.855$, $p < 0.001$), number of leaves ($F_{1,238} = 13.941$, $p < 0.001$), number of stems ($F_{1,238} = 14.658$, $p < 0.001$), LPR ($F_{1,238} = 13.898$, $p < 0.001$), and SER ($F_{1,238} = 12.359$, $p = 0.001$). For all individual traits with significant interactions, elevated temperature was associated with increased mean values in *P. graminifolia* (Figure 2). In contrast, elevated temperature did not significantly influence the mean values of most growth traits or biomass in *P. ruthii*; however, the number of stems of *P. ruthii* decreased significantly with elevated temperature (Figure 2).

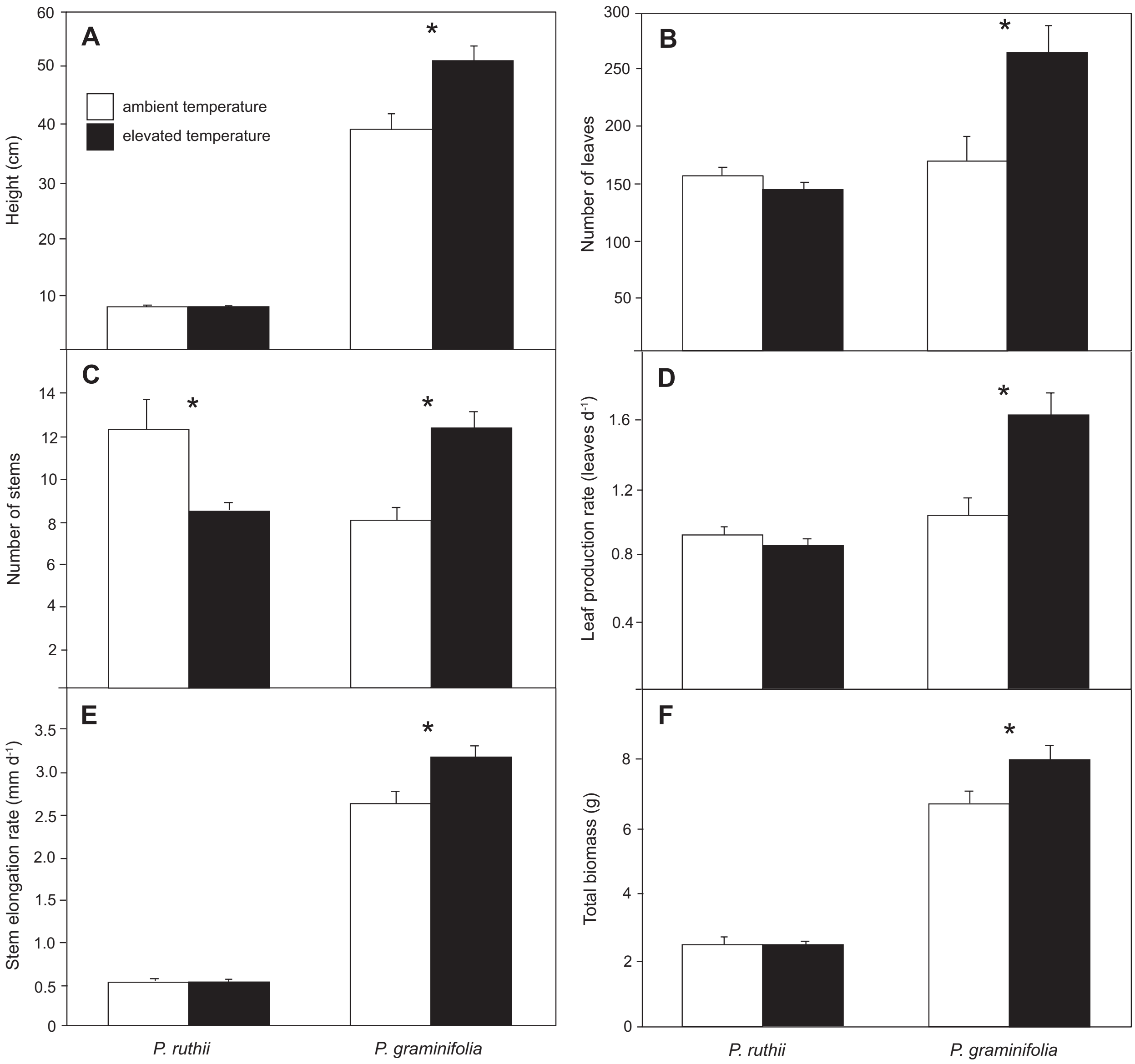

Figure 2 Mean height (**A**), number of leaves (**B**), number of stems (**C**), leaf production rate (**D**), stem elongation rate (**E**), and total dry biomass (**F**) of individuals of rare *Pityopsis ruthii* and common *P. graminifolia* grown from field-collected seed in ambient temperature (20-30°C night-day) and in elevated temperature (22-32°C night-day). Error bars represent 1 SE of the mean. Asterisks denote significant differences between mean values in ambient and elevated temperature within species at the $p \leq 0.05$ significance level

The interaction of species × water on growth, allocation, and total biomass of *P. ruthii* and *P. graminifolia* was significant (Table 4 and 5). Of individual allocation traits, only RLR was influenced significantly by the species × water interaction ($F_{1,245} = 6.385$, $p = 0.012$). Growth traits significantly influenced by this interaction included number of leaves ($F_{1,245} = 8.244$, $p = 0.004$) and number of stems ($F_{1,245} = 10.931$, $p = 0.001$), and LPR ($F_{1,245} = 7.862$, $p = 0.005$). For all individual traits with significant interactions, increased water influenced *P. ruthii* but not *P. graminifolia* (Figure 3). Specifically, increased water was associated with increased RLR, but decreased leaf number, stem number, LPR, and biomass (Figure 3).

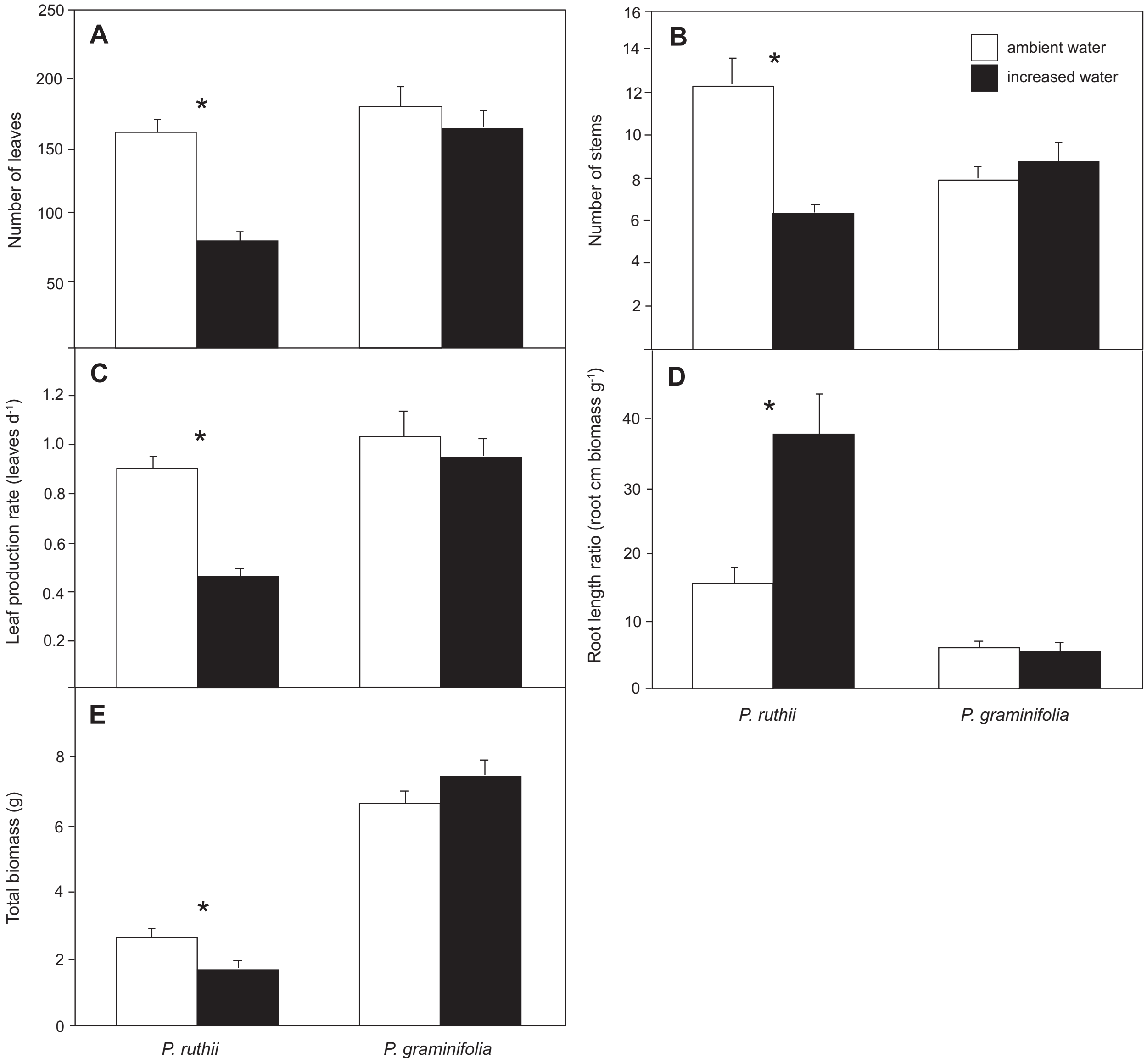

Figure 3 Mean number of leaves (**A**), number of stems (**B**), leaf production rate (**C**), root length ratio (**D**), and total dry biomass (**E**) of individuals of rare *Pityopsis ruthii* and common *P. graminifolia* grown from field-collected seed with ambient water availability (50% field capacity) and increased water availability (100% field capacity). Error bars represent 1 SE of the mean. Asterisks denote significant differences between mean values in ambient and elevated temperature within species at the $p \leq 0.05$ significance level

Phenotypic trait plasticity

Plasticity of growth and allocation in response to modified light differed significantly between *P. ruthii* and *P. graminifolia* (Table 5). Plasticity of height and SER were significantly lower in *P. ruthii* than *P. graminifolia*, while *P. ruthii* exhibited greater plasticity of SMF than did *P. graminifolia* (Table 6). Plasticity of both height ($F_{3,45} = 12.412$, $p < 0.001$) and SER ($F_{3,45} = 14.424$, $p < 0.001$) also differed significantly among distinct occurrences of *P. graminifolia*, with the GA2 occurrence exhibiting significantly greater plasticity of both variables than the other three occurrences; however plasticity of SMF did not differ between *P. graminifolia* occurrences ($F_{3,45} = 1.518$, $p = 0.217$). Similarly, there were no differences between *P. ruthii* occurrences for plasticity of height ($F_{3,73} = 3.915$, $p = 0.492$), SER ($F_{3,73} = 0.073$, $p = 0.403$), or SMF ($F_{3,73} = 2.650$, $p = 0.733$).

Table 6 Relative distances plasticity index (RDPI) values of growth and allocation traits and specific leaf area (SLA) and instantaneous leaf-leaf photosynthesis rate (*A*) of rare *Pityopsis ruthii* and common *P. graminifolia*. Units of measure are listed in Table 2. Values shown are means ± 1SE of the mean. Asterisks denote significant differences in species means at the $p \leq 0.05$ significance level

	Light		**Temperature**		**Water**	
RDPI trait	***P. ruthii***	***P. graminifolia***	***P. ruthii***	***P. graminifolia***	***P. ruthii***	***P. graminifolia***
Growth						
Height	0.13±0.01	0.20±0.03*	0.10±0.01	0.22±0.03*	0.14±0.02	0.19±0.02
Leaves	0.34±0.02	0.40±0.04	0.24±0.02	0.36±0.04*	0.39±0.03	0.33±0.04
Stems	0.34±0.03	0.33±0.03	0.26±0.02	0.33±0.03*	0.32±0.03	0.28±0.03
LPR	0.34±0.02	0.40±0.04	0.25±0.02	0.35±0.04*	0.38±0.03	0.32±0.03
SER	0.12±0.01	0.19±0.02*	0.12±0.01	0.18±0.02*	0.15±0.01	0.15±0.02
RL	0.18±0.02	0.17±0.02	0.17±0.02	0.15±0.02	0.20±0.02	0.16±0.02
Allocation						
RSR	0.31±0.03	0.26±0.03	0.27±0.03	0.31±0.03	0.41±0.03	0.26±0.03*
SMF	0.20±0.03	0.12±0.02*	0.17±0.02	0.14±0.02	0.29±0.02	0.12±0.02*
RMF	0.15±0.02	0.16±0.02	0.12±0.02	0.19±0.02*	0.17±0.02	0.16±0.03
SRL	0.41±0.03	0.38±0.04	0.39±0.03	0.43±0.04	0.40±0.03	0.41±0.04
RLR	0.37±0.03	0.35±0.04	0.34±0.03	0.39±0.04	0.41±0.03	0.34±0.04
Leaf morphology						
SLA	0.16±0.01	0.16±0.02	0.17±0.02	0.15±0.02	0.15±0.02	0.17±0.03
Leaf physiology						
A	0.27±0.02	0.24±0.03	0.18±0.02	0.31±0.04*	0.26±0.03	0.35±0.04

Within the context of elevated temperature, plasticity of growth, allocation, and leaf *A* differed significantly between species (Table 5). Plasticity of all measured growth traits with the exception of RL was significantly less in *P. ruthii* than in *P. graminifolia* (Table 6). Similarly, *P. ruthii* was characterized by less plasticity of both RMF and leaf *A* than *P. graminifolia* (Table 6). There were no differences among occurrences of either species in any of the plasticity measures that differed between species.

Plasticity of growth and allocation in response to altered water availability differed significantly between *P. ruthii* and *P. graminifolia* (Table 5). Although there were no significant differences in the plasticity of individual growth traits between species, plasticity of both RSR and SMF as allocation traits was significantly greater in *P. ruthii* than in *P. graminifolia* (Table 6). Among *P. ruthii* occurrences, there were significant differences in the plasticity of SMF ($F_{3,72} = 3.327$, $p = 0.022$) with the O1 occurrence exhibiting significantly greater plasticity of this variable than the other three occurrences of this species; however, plasticity of RSR did not differ between *P. ruthii* occurrences ($F_{3,72} = 1.242$, $p = 0.297$). Similarly, there were no differences between *P. graminifolia* occurrences for plasticity of SMF ($F_{3,45} = 0.618$, $p = 0.605$) or RSR ($F_{3,73} = 0.480$, $p = 0.697$).

There were numerous significant associations between trait plasticity and total biomass (as a fitness proxy) in both *P. ruthii* and *P. graminifolia* (Table 7). For *P. ruthii*, these associations were positive within the context of light and negative within the context of water. When assessed across temperature conditions, total biomass was negatively associated with plasticity of growth traits but positively associated with allocation traits in *P. ruthii* (Figure 4). For *P. graminifolia*, significant associations between trait plasticity and total biomass were consistently negative within the context of light, temperature, and water (Figure 5).

Table 7 Results of linear regression analysis to test for the significance of associations between trait plasticity assessed with a relative distances plasticity index (RDPI) in response to differences in light, temperature, and water conditions and total dry biomass (as a fitness proxy) of rare *Pityopsis ruthii* and common *P. graminifolia* individuals. Asterisks denote *p*-values significant at ≤ 0.05 significance level

		Light		**Temperature**		**Water**	
Species	**RDPI trait**	r^2	p	r^2	p	r^2	p
P. ruthii	**Growth**						
	Height	0.021	0.283	0.059	0.061	0.081	0.020*
	Leaves	<0.001	0.979	0.101	0.014*	0.039	0.122
	Stems	0.120	0.008*	0.001	0.804	0.380	0.126
	LPR	0.001	0.752	0.126	0.005*	0.034	0.150
	SER	0.004	0.614	0.016	0.336	0.075	0.031*
	RL	0.003	0.659	0.001	0.744	0.027	0.195
	Allocation						
	RSR	0.091	0.023*	0.088	0.021*	0.006	0.526
	SMF	0.103	0.015*	0.113	0.009*	0.034	0.149
	RMF	0.047	0.106	0.013	0.384	0.009	0.451
	SRL	0.054	0.082	0.035	0.154	0.007	0.473
	RLR	0.036	0.158	0.017	0.313	0.029	0.185
	Leaf morphology						
	SLA	<0.001	0.976	0.007	0.577	0.072	0.080
	Leaf physiology						
	A	0.011	0.513	0.003	0.692	0.028	0.355
P. graminifolia	**Growth**						
	Height	0.044	0.224	0.017	0.445	0.032	0.279
	Leaves	0.097	0.068	0.039	0.247	0.033	0.272
	Stems	0.041	0.242	<0.001	0.918	0.024	0.352
	LPR	0.144	0.024*	0.013	0.500	0.058	0.144
	SER	0.093	0.073	<0.001	0.882	0.005	0.663
	RL	0.242	0.004*	0.003	0.721	0.019	0.414
	Allocation						
	RSR	0.004	0.686	0.134	0.027*	0.039	0.229
	SMF	0.026	0.347	0.151	0.019*	0.014	0.473
	RMF	<0.001	0.965	0.131	0.029*	0.058	0.144
	SRL	0.016	0.493	<0.001	0.960	0.084	0.112
	RLR	0.214	0.009*	0.045	0.216	0.169	0.011*
	Leaf morphology						
	SLA	<0.001	0.913	<0.001	0.992	0.012	0.505
	Leaf physiology						
	A	0.194	0.059	0.048	0.231	0.007	0.704

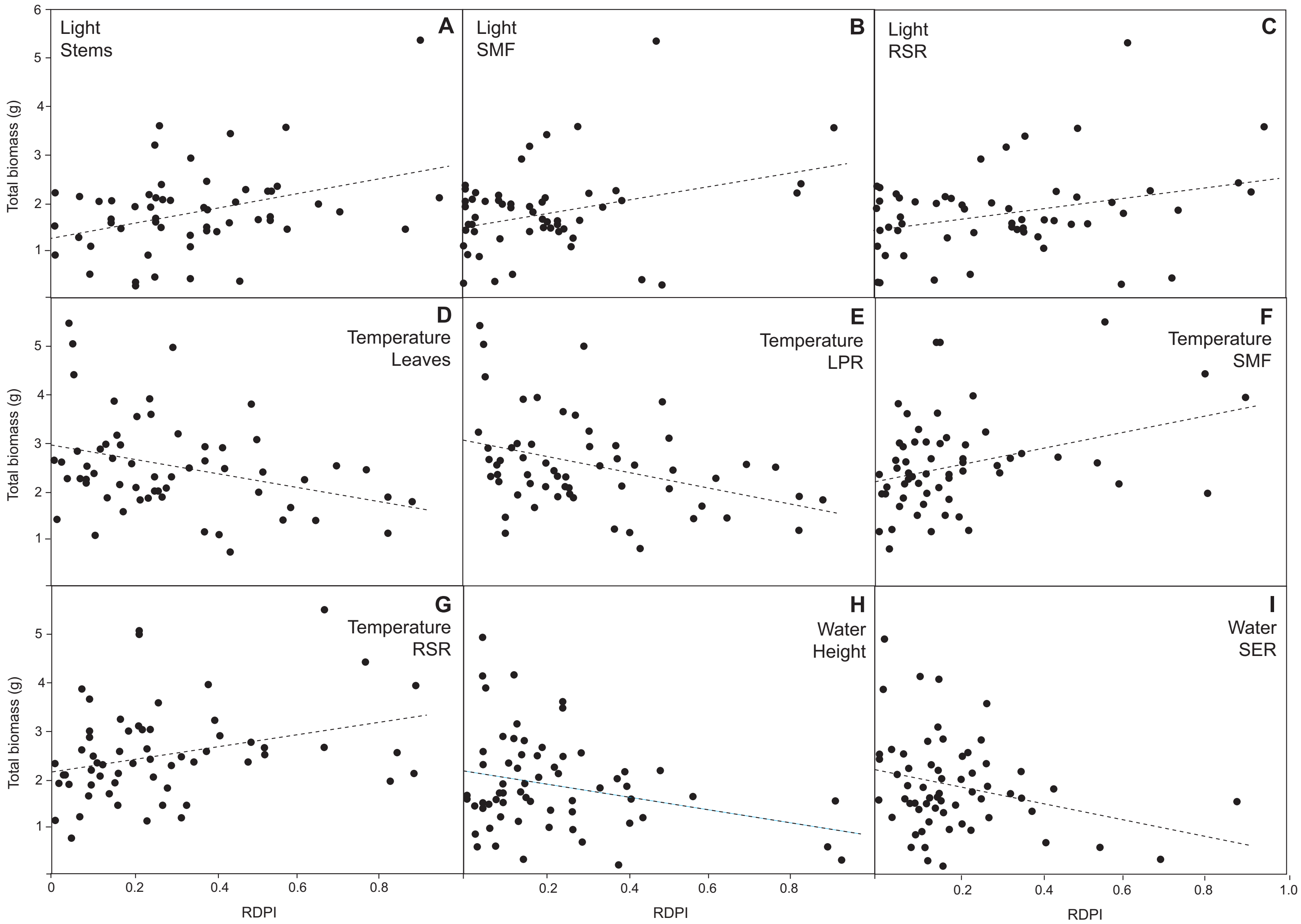

Figure 4 Linear regressions of depicting significant associations between trait plasticity in assessed with a relative distances plasticity index (RDPI) in response to differences in light (**A**, **B**, **C**, **D**), temperature (**E**, **F**, **G**, **H**), and water (**I**) conditions and total dry biomass (as a fitness proxy) of rare *Pityopsis ruthii*. Trait abbreviations are listed in Table 2. All regressions shown are significant at the $p < 0.05$ level

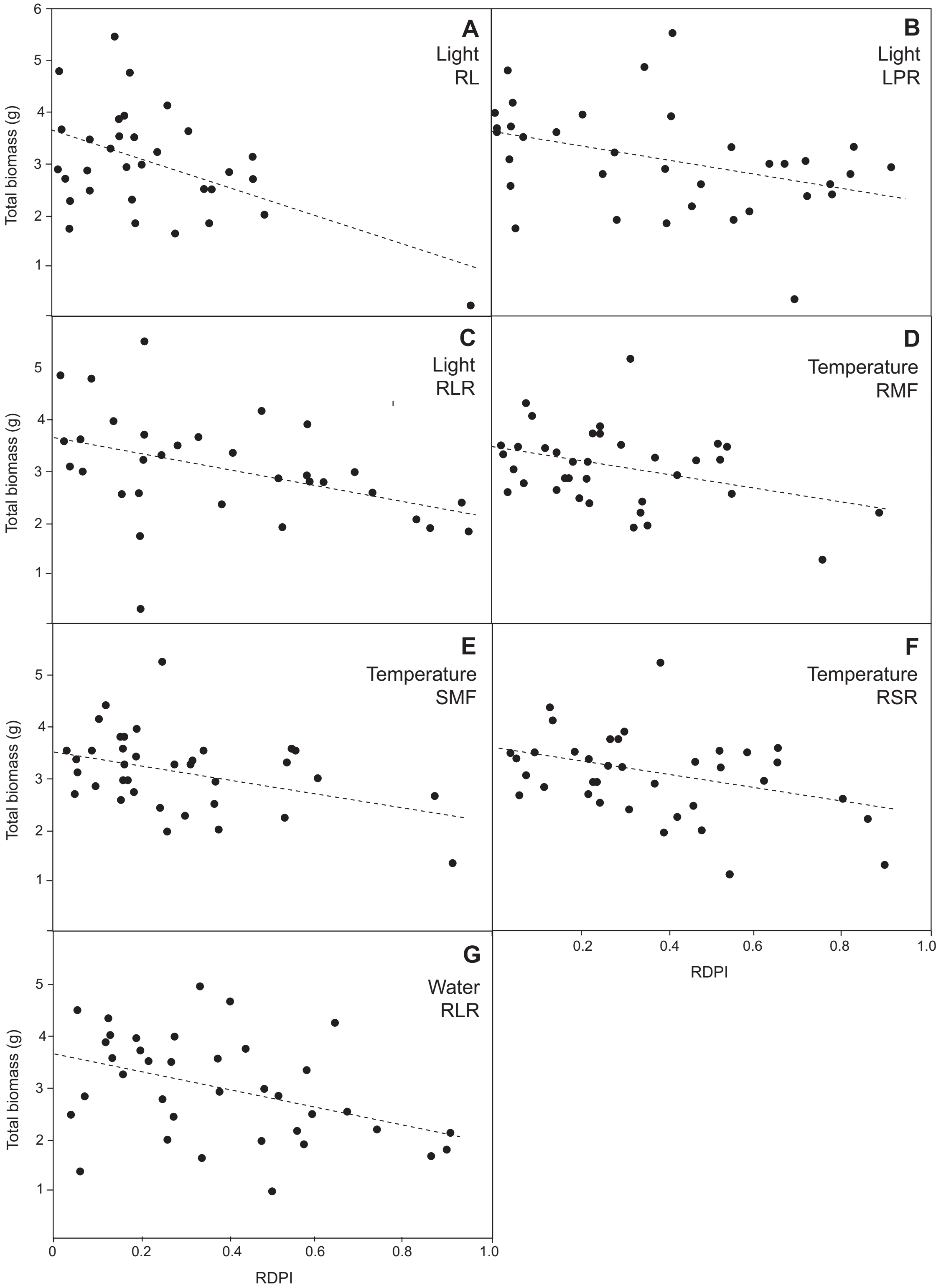

Figure 5 Linear regressions of depicting significant associations between trait plasticity in assessed with a relative distances plasticity index (RDPI) in response to differences in light (**A**, **B**, **C**), temperature (**D**, **E**, **F**), and water (**G**) conditions and total dry biomass (as a fitness proxy) of rare *Pityopsis graminifolia*. Trait abbreviations are listed in Table 2. All regressions shown are significant at the $p \leq 0.05$ level

Discussion

Comparative responses to environmental differences in* Pityopsis *species

Insights from recent research investigating intraspecific variability of trait values suggests that the ability to deviate from mean trait values may influence species abundance by enabling colonization of novel habitats throughout a species' range (Umaña et al. 2015). Furthermore, intraspecific trait variation may play an important role in how plants respond to anthropogenic changes (Henn et al. 2018, Norberg et al. 2001) with traits that exhibit low variation across different environments responding more slowly to a shift in ideal trait values (Henn et al. 2018). Each altered environmental condition influenced both the rare and common *Pityopsis* species' trait values in markedly different ways. The overall decrease in trait values for *P. ruthii* across altered conditions and the increase in trait values of *P. graminifolia* within increased temperature conditions exemplifies the relationship between greater intraspecific variation and increased abundance through success across numerous environment types.

Environmental conditions were purposely manipulated to reflect anthropogenic changes experienced by *P. ruthii* and thus allowed us to assess the potential responses and impacts of these alterations in comparison to its widespread congener. The results of decreased light availability are validated by previous assessments of *P. ruthii* describing a species adapted to grow best in high light conditions that may be negatively impacted by the shade of encroaching competitors (Moore 2016, Thompson and Schwartz 2006). While observed responses to temperature are supported by research suggesting a significant alteration in the physiological responses of rare species to temperature change and ultimately predicting a greater vulnerability of endemic species than generalists to a continued rise in temperature (Jeong et al. 2018). Our results imply that while *P. graminifolia* may fare well in a warming climate, *P. ruthii* may not. The response to water availability is most relevant to persistence of *P. ruthii* as it is often subjected to drought, inundation, and scouring events historically and as a result of continued river management (Thomson and Schwartz 2006, Moore et al. 2016). Although previous studies have found a persistence of *P. ruthii* individuals under periodic inundation, more recent evidence gathered by the TVA seems to suggest any extended periods of consistently elevated water levels may be potentially harmful to the species (A. Dattilo unpublished report 2019). Decreased trait values seem to corroborate the findings of the TVA while the increase in root length ratios suggests that in response to extended inundation *P. ruthii* may expand its root system possibly to anchor itself deeper into the boulder crevices.

Based on our results we can assume that *P. ruthii* will experience negative growth and productivity consequences while *P. graminifolia* will experience no change or a potentially increased growth pattern in terms of response to changing conditions. Considered collectively, the results of both *Pityopsis* species' trait values indicate an intraspecific trait variability that is primarily dependent on response to resource availability. Additionally, their responses to altered conditions/resources are reflective of how rare and common species might respond to future anthropogenic change suggesting a potential series of consequences for *P. ruthii* especially with regards to increased water levels. In comparison, we can speculate that although altered light and water seem to have no effect on *P. graminifolia* increased temperatures may lead to greater growth and productivity.

Comparative plasticity of* Pityopsis *species

Understanding the biological factors underlying species rarity and commonness and the responses of these factors to environmental change has vital implications for ecological theory as it relates to inherent differences in species prevalence (Bevill and Louda 1999). Knowledge of plasticity may have an outsized role in rare species conservation, highlighted by the rapid environmental change threatening rare species in particular (Mouillot et al 2013). Plasticity may allow for acclimation to change without the required genetic changes of adaptation, a fairly long-term process that may be inhibited by the low genetic diversity of many rare species (Leimu 2008). Ecophysiological trait plasticity, in particular, has been shown to have an especially fast response time to environmental change relative to other types of traits (Funk et al. 2007). Thus, it is feasible that plasticity could buffer negative impacts of environmental change in the short-term, potentially allowing time for genetic adaptations to evolve (Jump and Peñuelas 2005). Yet, to our knowledge, the results of the broader body of literature comprised primarily of autoecological studies comparing the biological traits of rare and common species has not been comprehensively reviewed in nearly 20 years (Murray et al 2002) and, as a consequence, the link between traits, plasticity, and species' prevalence remains a knowledge gap in the field of ecology (Umaña et al. 2015).

Plants are signified by high degrees of trait plasticity (Sultan 2000) and plant species and populations can vary dramatically in their responses to environmental change due to plasticity differences (Osunkoya & Swanborough 2001, Cleavittt 2002, Pohlman et al. 2005, Dangremond et al. 2015, Nicotra & Davidson 2010, Godoy et al. 2012, Sultan 2000, Schlichting & Levin 1984, Valladares et al. 2000, Balaguer et al. 2001, Valladares et al. 2007). Several previous studies have intimated the potential importance of rarity and plasticity in terms of persistence and dynamics of habitat specialists (See Review by: Murray et al. 2002). The results of our plasticity comparisons suggest that rare plant species may exhibit less plasticity of traits than their common congeners. Each altered environmental condition affected the plasticity indices of each species differently and plasticity was similarly influenced by resource dependence much like the responses of the mean trait values.

Our results suggest that the ability for either *P. ruthii* or *P. graminifolia* to buffer the effects of change is dependent on the type of change the species experiences and what traits are most important to its persistence. With a reduction in light *P. graminifolia* might fare slightly better but only in terms of growth plasticity while *P. ruthii* could alter its allocation of shoot mass. Increased temperature conditions clearly favor *P. graminifolia* with a range of traits observed to have greater plasticity, all but ensuring this species will thrive with the arrival of higher temperatures while *P. ruthii* struggles to acclimate. Increased water availability as a condition favors a greater plastic response in *P. ruthii* allocation traits suggesting that, although this species may initially perform poorly in terms of physical response, the increased plasticity will allow potential time to acclimate with regard to how biomass is allocated below or aboveground. Trait type (i.e. growth, allocation, leaf level) also plays a part in plasticity with rare species individuals exclusively displaying greater plasticity in allocation traits only and common species exhibiting plasticity predominantly in growth, but also allocation and leaf level traits. These plasticity differences help explain the habitat specificity of *P. ruthii*, in terms of light constraints and ability to manage dynamic water regimes, as well as the species reduced distribution potential in comparison to *P. graminifolia*.

As a result of anthropogenic changes, organisms with long generations and constrained dispersal, such as endemic perennial plants like *P. ruthii*, may be in need of rapid phenotypic adaptation (Lande 2009). Phenotypic adaptation occurs either through evolution by natural selection or the associated genetic changes of phenotypic plasticity brought on by environmental change (Lande 2009). In regard to fitness, phenotypic plasticity is often thought of as adaptive, but can also be maladaptive or neutral. Adaptive plasticity, which offers a fitness benefit, is most likely to facilitate quick adaptation to altered environmental conditions, however, maladaptive plasticity can also fuel adaptative evolution by revealing cryptic genetic variation that may result in a more fit phenotype (Nicotra et al. 2010, Ghalambor et al. 2007). In this instance, maladaptive plasticity to environmental change increases the variance and by chance a subset of individuals exhibits a beneficial response that is passed on through reproduction allowing adaptation to occur (Ghalambor et al. 2007). Therefore, both maladaptive and adaptive plasticity are likely to play a significant part in how plants respond to anthropogenic changes in the future (Nicotra et al. 2010).

At the very least our results suggest that greater plasticity under certain traits/conditions increases the fitness of *P. ruthii* individuals while others decrease fitness. Although this again was dependent on the trait type as well as the environmental change experienced with negative associations demonstrated under growth and leaf traits, and different resource availability. In contrast, *P. graminifolia* individuals experience a decreased level of fitness with all greater plasticity across traits and conditions. In short, plasticity is more likely to benefit *P. ruthii* than *P. graminifolia* in terms of fitness. This plasticity advantage implies that distribution of *P. ruthii* may be constrained by a lack of plasticity among most wild individuals. Although additional research into the potential of maladaptive plasticity to produce new phenotypes for both species should be investigated. Furthermore, it should be noted that our results may be potentially skewed as we used total biomass as a proxy for fitness in opposition to other studies which used more relevant fitness measures such as flower number or seed production.

Implications for **P. ruthii** *persistence in the face of environmental change*

As anthropogenic changes continue to impact *P. ruthii* the species will be undoubtedly affected by a range of consequences. Occurrences will be subjected to altered light and water availability as well as increased temperatures that will likely influence relative abundance and further constrain distribution potential. Individuals may respond to these changes with less growth and productivity reducing the future viability of the species. While plasticity might allow for buffering of environmental change under certain conditions this is reduced specifically to allocation traits which may not be sufficient to protect *P. ruthii* in the long term. Furthermore, the perceived lack of plasticity among individuals is problematic to ensuring the mechanism allows for adaptation within the species. As evidenced by the precipitous drop in individual numbers during TVA inundation events (A. Dattilo, unpublished report 2019) we can expect a similar decline as this and other impacts continue to play out. Without the time to adapt nor the ability to acclimate in the short-term *P. ruthii* faces an uncertain future and will require supplemental assistance to ensure its continued survival.

Management recommendations

Based on our findings, we have made several recommendations for the continued management of *P. ruthii* that may help the species persist and avoid potential extinction risks in response to expected anthropogenic changes. In regard to light alteration, we suggest the sustained control of encroaching woody vegetation involving the removal of any competitor species that have become establish near any vulnerable occurrences of *P. ruthii* as the species has continually proven to be adversely affected by increased shading. Further, continued assessments of changes in encroaching vegetation should be made during the annual monitoring efforts for the species. While little can be done to mitigate the changes in temperature increase expected to negatively influence *P. ruthii* we recommend including tracking these changes by measuring temperature fluctuations during monitoring. Each individual occurrence should be assessed for temperature change affects as different occurrences may respond in varied ways with special attention given to reduced stem number as a potential sign of negative temperature effects. Along both the Hiwassee and Ocoee Rivers extended periods of inundation and other altered flows are some of the most pressing issues for the TVA and its management of *P. ruthii*. Having confirmed the hazardous potential of long-term inundation we recommend avoiding it at all costs as increased water levels may cause a variety of issues that occurrences may not be able to recover from. Obviously, this is not always avoidable so if altered flows are required, say in the event of a dam closure, we instead recommend short periods of inundation or high flow releases that the species is accustomed to. If for some reason long term inundation is completely unavoidable, we also recommend collecting seed or cuttings to propagate individuals for transplantation back to the affected areas once waters have receded. As a last resort, we suggest moving occurrences of *P. ruthii* threatened by environmental change but only if there are no other options as translocation of the species has proven to be quite unsuccessful. Finally, although the Ocoee1 occurrence was significantly more plastic than all other occurrences for one trait in one altered condition this is not enough information to suggest changes in how to manage either rivers differently. Indeed, the Hiwassee River occurrences perform much better on average than those along the Ocoee River suggesting some unknown aspect affecting their abundances.

In this context we would recommend continued research into the differences between occurrences on each river, specifically with a focus on genetic variation, to determine the ways in which these occurrences differ and how to best manage them in the future.

REFERENCES

Baskauf CJ, Eickmeier WG. (1994) Comparative ecophysiology of a rare and a widespread species of *Echinacea* (Asteraceae). *American Journal of Botany* 81:958-964.

Baskin JM, Snyder KM, Walck JL, Baskin C. (1997) The comparative autecology of endemic, globally-rare, and geographically-widespread, common plant species: three case studies. *The Southwestern Naturalist* 42:384-399.

Bevill RL, Louda SM. (1999) Comparisons of related rare and common species in the study of plant rarity. *Conservation Biology* 13:493-498.

Blackburn TM, Jeschke JM. (2009) Invasive success and threat status: two sides of a different coin? *Ecography* 32:83-88.

Bradshaw CJA, Giam XL, Tan HTW, Brook BW, Sodhi NS. (2008) Threat or invasive status in legumes is related to opposite extremes of the same ecological and life-history attributes. *Journal of Ecology* 96:869-883.

Cadotte MW, Murray BR Lovett-Doust. (2006) Ecological patterns and biological invasions: using regional species inventories in macroecology. *Biological Invasions* 8:809-821.

Cleavitt NL. (2002) Stress tolerance of rare and common moss species in relation to their occupied environments and asexual dispersal potential. *Journal of Ecology* 90:785-795.

Cole CT. (2003) Genetic variation in rare and common plants. *Annual Review of Ecology, Evolution, and Systematics* 34: 213-237.

Combs JK, Lambert AM, Reichard SH. (2013) Predispersal seed predation is higher is a rare species than in its widespread sympatric congeners (*Astragalus*, Fabaceae). *American Journal of Botany* 100:2149-2157.

Daehler CC. (2003) Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. *Annual Review of Ecology, Evolution, and Systematics* 34:183-211.

Dangremond EM, Feller IC, Sousa WP. (2015) Environmental tolerances of rare and common mangroves along light and salinity gradients. *Oecologia* 179:1187-1198.

Darwin CR. (1859) *On the Origin of Species.* John Murray, London.

Davidson AM, Jennions M, Nicotra AB. (2011) Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters* 14:419-431.

Denton MD, Venklaas E, Lambers H. (2007) Does phenotypic plasticity in carboxylate exudation differ among rare and widespread *Basksia* species (Proteaceae)? *New Phytologist* 173:592-599.

Farnsworth EJ. (2006) Plant life history traits of rare versus frequent plant taxa of sandplains: implications for research and management trials. *Biological Conservation* 136:44-52.

Funk JL. (2008) Differences in plasticity between invasive and native plants from a low resource environment. *Journal of Ecology* 96:1162-1173.

Funk JL, Jones CG, Lerdau MT (2007) Leaf- and shoot-level plasticity in response to different nutrient and water availabilities. *Tree Physiology* 27:1731-1739.

Gibson DJ. (2014) *Methods in Comparative Plant Ecology*. Oxford University Press, UK, pp. 320

Gitzendanner MA, Soltis PS. (2000) Patterns of genetic variation in rare and widespread plant congeners. *American Journal of Botany* 87:783-792.

Godoy O, Valladares F, Castro-Díez P. (2012) The relative importance for plant invasiveness of trait means, and their plasticity and integration in a multivariate framework. *New Phytologist* 195:912-922.

Godt MJ, Hamrick WJL. (2001) Genetic diversity in rare Southeastern plants. *Natural Areas Journal* 21:61-70.

Henn, JJ, Buzzard, V, Enquist, BJ, Halbritter, AH, Klanderud, K, Maitner, BS, Michaletz, ST, Pötsch, C, Seltzer, L, Telford, RJ, Yang, Y (2018) Intraspecific trait variation and phenotypic plasticity mediate alpine plant species response to climate change. *Frontiers in Plant Science* 9:1548-1559.

Kruckeberg AR, Rabinowitz D. (1985) Biological aspects of endemism in higher plants. *Annual Review of Ecology and Systematics* 16:447-479.

Kunin WE, Gaston K. (1997) *The Biology of Rarity: Causes and Consequences of Rare-Common Differences*. Springer, New York, NY, USA.

Lavergne S, Thompson JD, Garnier E, Debussche M. (2004) The biology and ecology of narrow endemic and widespread plants: a comparative study of trait variation in 20 congeneric pairs. *Oikos* 107:505-518.

Leffler AJ, James JJ, Monaco TA, Sheley RL. (2014) A new perspective on trait differences between native and invasive exotic plants. *Ecology* 95:298-305.

Leimu R, Fischer M (2008) A meta-analysis of local adaptation in plants. *PLOS One* 3: e4010.

Liu JC, Temme AA, Cornwell WK, van Logtestijn RS, Aerts R, Cornelissen JH. (2016) Does plant size affect growth responses to water availability at glacial, modern and future CO 2 concentrations? *Ecological Research* 31:213-227.

Lovell JT, McKay JK. (2015) Ecological genetics of range size variation in *Boechera* spp. (Brassicaceae). *Ecology and Evolution* 5:4962-4975.

Marchin RM, Bhandari RK, Wall WA, Hohmann MG, Gray JB, Hoffman WA. (2009) Are rare species less shade tolerant than common species in fire-prone environments? A test with seven *Amorpha* (Fabaceae) species. *Plant Ecology* 205:249-260.

May RM. (1988) How many species are there on earth? *Science* 241:1441-1449.

May R. (1999) Unanswered questions in ecology. *Philosophical Transactions of the Royal Society of London* 354:1951-1959.

McGill BJ. (2006) A renaissance in the study of abundance. *Science* 314:770-772.

Moore PA, Wadl PA, Skinner JA, Trigiano RN, Bernard EC. (2016) Current knowledge, threats, and future efforts to sustain populations of *Pityopsis ruthii* (Asteraceae), an endangered southern Appalachian species. *Journal of the Torrey Botanical Society* 143:117-134.

Mouillot D, Bellwood DR, Baraloto C, Chave J, Galzin R, Harmelin-Vivien M, Kulbicki M, Lavergne S, Lavorel S, Mouquet N, Paine CET, Renaud J, Thuiller W. (2013) Rare species support vulnerable functions in high-diversity ecosystems. *PLOS Biology* 11:e1001569.

Murray BR, Thrall PH, Gill M, Nicotra A. (2002) How plant life-history and ecological traits relate to species rarity and commonness at varying spatial scales. *Austral Ecology* 27:291-310.

National Oceanic and Atmospheric Administration. (2018) Data tools: Summary of Monthly Normals 1981–2010; Copperhill, TN Station. *National Centers for Environmental Information*. https://www.ncdc.noaa.gov/cdo-web/quickdata. Accessed Jan 2018.

NatureServe. (2009) Pityopsis ruthii (Small) Small. *Natureserve explorer: an online* *encyclopedia of life*. https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.153865/Pityopsis_ruthii. Accessed Feb 2018.

Nicotra AB, Davidson A. (2010) Adaptive phenotypic plasticity and plant water use. *Functional Plant Biology* 37:117-127.

Osunkoya OO, Swanborough PW. (2001) Reproductive and ecophysiological attributes of the rare *Gardenia actinocarpa* (Rubiaceae) compared with its common co-occurring congener, *G-ovularis*. *Australian Journal of Botany* 49:471-478.

Palacio-López K, Gianoli E. (2011) Invasive plants do not display greater phenotypic plasticity than their native or non-invasive counterparts: a meta-analysis. *Oikos* 120:1393-1401.

Pandit MK, Pocock MJO, Kunin WE. (2011) Ploidy influences rarity and invasiveness in plants. *Journal of Ecology* 99:1108-1115.

Palumbi SR. (2001) *The evolution explosion: how humans cause rapid evolutionary change.* WW Norton & Company, New York.

Pohlman CL, Nicotra AB, Murray BR. (2005) Geographic range size, seedling ecophysiology and phenotypic plasticity in Australian *Acacia* species. *Journal of Biogeography* 32:341-351.

Pyŝek P, Richardson DM. (2007) Traits associated with invasiveness in alien plants: where do we stand? *In* Nentwig W (ed) *Biological Invasions, Ecological Studies* 193:97-126.

Rabinowitz D. (1981) Seven forms of rarity. *In* Synge H (ed) The Biological Aspects of Rare Plant Conservation, John Wiley & Sons Ltd, Hoboken, NJ, USA, pp 205-217.

Ruprecht E, Fenesi A, Nijs I. (2014) Are plasticity in functional traits and constancy in performance traits linked with invasiveness? An experimental test comparing invasive and naturalized plant species. *Biological Invasions* 16:1359-1372.

Rünk K, Zobel K. (2007) Phenotypic plasticity and biomass allocation pattern in three *Dryopteris* (Dryopteridaceae) species on an experimental light-availability gradient. *Plant Ecology* 193:85-99.

Schmidt JP, Stephens PR, Drake JM. (2012) Two sides of the same coin? Rare and pest plants native to the United States and Canada. *Ecological Applications* 22:1512-1525.

Schulze ED, Ellis R, Schulze W, Trimborn P. (1996) Diversity, metabolic types, and delta C-13 carbon isotope ratios in the grass flora of Namibia in relation to growth form, precipitation and habitat conditions. *Oecologia* 106:352-369.

Semple, JC. (2006) Pityopsis Nuttall. *Flora of North America* 20:222-226.

Stebbins GL. (1942) The genetic approach to problems of rare and endemic species. *Madroño* 6:241-258.

Thomson DM, Schwartz MW. (2006) Using population count data to assess the effects of changing river flow on an endangered riparian plan. *Conservation Biology* 20:1132-1142.

Umaña MN, Zhang C, Cao M, Lin L, Swenson NG (2015) Commonness, rarity, and intra-specific variation in traits and performance in tropical tree seedlings. *Ecology Letters* 18:1329-1337.

United States Fish and Wildlife Service (USFWS). (2015) Endangered and threatened wildlife and plants; threatened species status for Pityopsis ruthii (Ruth's Golden Aster). *Fed Reg* 50: 29341.

Vanderhoeven S, Brown CS, Tepolt CK, Tsutsui ND, Vanparys V, Atkinson S, Mahy G, Monty A. (2010) Linking concepts in the ecology and evolution of invasive plants: network analysis shows what has been most studied and identifies knowledge gaps. *Evolutionary*

Applications 3:193-202.

van Kleunen M, Fischer M. (2009) Release from foliar and floral fungal pathogen species does not explain the geographic spread of naturalized North American plants in Europe. *Journal of Ecology* 97:385-392.

van Kleunen M, Weber W, Fischer M. (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters* 2:235-245.

Wadl PA, Rinehart TA, Dattilo, AJ, Pistrang M, Vito LM, Milstead R, Trigiano RN. (2014). Propagation for the conservation of Pityopsis ruthii, an endangered species from the southeastern United States. *HortScience* 49:194-200.

Wiegand TP, Gentry B, McCoy Z, Tannis C, Klug H, Bonsall MB, Boyd JN (2020) Visualizing connectivity of ecological and evolutionary concepts – an exploration of research on plant species rarity. *Ecology and Evolution* 10:9037-9047.

VITA

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