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Traits of an invasive grass conferring an early growth advantage over native grasses

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Abstract

Aims

Invasive species often have higher relative growth rates (RGR) than their native counterparts. Nutrient use efficiency, total leaf area and specific leaf area (SLA) are traits that may confer RGR differences between natives and invasives, but trait differences are less prominent when the invasive species belongs to the same plant functional type as the dominant native species. Here, we test if traits displayed soon after germination confer an early size advantage. Specifically, we predicted that invasive species seedlings grow faster than the natives because they lack trade-offs that more strongly constrain the growth of native species.

Methods

We quantified plant morphological and physiological traits and RGR during early seedling growth at high and low nutrient levels in three dominant perennial native C_4 grasses: *Panicum virgatum* L. (switchgrass), *Schizachyrium scoparium* (Michx.) Nash (little bluestem) and *Andropogon gerardii* Vitman (big bluestem); and a perennial C_4 exotic invasive grass, *Sorghum halepense* (L.) Pers. (Johnsongrass).

Important Findings

After 2 weeks of growth, Johnsongrass seedlings had greater biomass, SLA and photosynthetic nitrogen use efficiency, but lower leaf N concentrations (% leaf N) and root:shoot ratio than natives. As growth continued, Johnsongrass more quickly produced larger and thicker leaves than the natives, which dampened the growth advantage past the first 2 to 3 weeks of growth. Investment in carbon gain appears to be the best explanation for the early growth advantage of Johnsongrass. In natives, growth was constrained by an apparent trade-off between allocation to root biomass, which reduced SLA, and production of leaves with high N content, which increased carbon gain. In Johnsongrass, root:shoot ratio did not interact with other traits, and % leaf N was decoupled from RGR as a result of a trade-off between the positive indirect association of % leaf N with RGR and the negative direct association of % leaf N with RGR.

Keywords: biomass allocation, invasive plants, RGR, *Sorghum halepense*, trade-offs, trait development

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INTRODUCTION

Exotic plant species invasions affect terrestrial ecosystems in many ways. Documented impacts include biodiversity loss (Sala *et al.* 2000), changes in nutrient pools (Hickman *et al.* 2010) and alteration of disturbance regimes (D'Antonio and Vitousek 1992). One reason for the success of some invasive species may lie in a competitive advantage arising from higher relative growth rates (RGR) than natives. Many researchers expect that certain trait differences between the invasive species and resident community members should explain differences in RGR and the competitive success of invasive species (James and Drenovsky 2007). When an invasive species differs greatly in life cycle and morphology from resident species in the community, e.g. when a shrub species invades a rangeland (Archer 1994, Throop *et al.* 2012), critical trait differences may be well defined and directly related to exploiting niches left vacant by the grassland community. However, when the invasive and the dominant resident species belong to the same functional group, the invasive and resident species would be expected to occupy similar niches (Chesson 2000), and express similar traits within the plant community. In these cases, the trait differences responsible for invasiveness become less clear. Invasiveness then may be tied to traits enabling resource pre-emption through an accelerated schedule of seedling emergence and growth (Martin *et al.* 2010, Wainwright *et al.* 2012).

When resource pre-emption becomes the competitive strategy, traits expressed during early phases of plant growth may be most critical, because they should provide an early size advantage that is likely to be maintained until maturity (Gerry and Wilson 1995; Hely and Roxburgh 2005; Pattison *et al.* 1998). Plant traits thought to correlate with faster seedling growth include higher total leaf area and SLA compared to natives (Allred *et al.* 2010; Dohleman *et al.* 2009; James and Drenovsky 2007; Smith and Knapp 2001). Invasives may also have lower leaf nitrogen requirements or higher nitrogen use efficiencies (Burke and Grime 1996; Funk and Vitousek 2007; Huenneke *et al.* 1990). Ultimately, a higher RGR allows invasives to capture more resources, enhance competitive effects and reduce the time to reproduction compared to natives.

Studies that focus on just one trait, such as root:shoot ratio or SLA, often fail to isolate the causes of invasiveness. For example, the invasion success of ~400 naturalized species in the German flora was better explained by a combination of traits including ploidy, shoot length and phenology than by the values of any single trait (Küster *et al.* 2008). Alien and native species typically have many trait differences, which do not vary or function independently, but are part of a comprehensive life history strategy (Grassein *et al.* 2015; Roberts *et al.* 2010; Westoby *et al.* 2002). An environment with herbivore pressure and low resource availability may select for increased allocation to roots and herbivore-resistant leaves, but these traits are often inversely related to SLA. Although together these traits may be advantageous in terms of plant fitness in nutrient-poor environments, the trade-off between root allocation and SLA may lower RGR (Reich 2014), which can be a liability in competition with alien species evolved under different ecological circumstances. Thus, different life histories may position invasive species at different points along multiple trade-off axes compared to the native flora (Adler *et al.* 2014; Leffler *et al.* 2014).

Here, we examined growth and multiple trait differences between native and invasive perennial C_4 grass species during early seedling growth. We quantified differences in seedling growth in three dominant, warm-season native perennial C_4 grasses; *Panicum virgatum* L. (switchgrass), *Schizachyrium scoparium* (Michx.) Nash (little bluestem) and *Andropogon gerardii* Vitman (big bluestem); and in the C_4 perennial, exotic invasive grass, *Sorghum halepense* (L.) Pers. (Johnsongrass). These native grasses are widespread and abundant throughout the Central Plains grasslands of North America (Coupland and Keeley 1994; Estes and Brunken 1982). Johnsongrass is native to the Mediterranean and now inhabits all warm-temperate regions worldwide. It was introduced to the USA in the early 1800s for forage, and has since invaded both native and agricultural ecosystems causing serious crop yield and economic losses (Warwick and Black 1983). Johnsongrass establishment is favored by disturbance or fertilization, and it persists by means of vigorous rhizome growth (Horowitz 1972; Horowitz 1973). However, its early seedling trait differences with native grasses have not been examined.

Our overall study objective was to determine the basis of RGR differences between seedlings of Johnsongrass and the native grasses. We tested first whether Johnsongrass seedlings grew faster than the native species when grown in monoculture. Next, we examined which traits or trait interactions explained differences in RGR between species. Among the traits we quantified for this purpose were root:shoot ratio, SLA, leaf nitrogen percentage by mass (% leaf N), leaf photosynthetic rates and several other related traits. Further, we tested whether the growth disparity between species increased with higher nutrient availability. We predicted that, compared to the native species, Johnsongrass would express (i) higher RGR, (ii) have a distinct trait ensemble with quantitatively different relationships between RGR and trait values, indicative of different rate limiting processes. Specifically, we expected natives to show trade-offs between traits governing the rate of nitrogen uptake (e.g. by root:shoot ratio) and traits governing the rate of carbon uptake (e.g. photosynthetic rates, SLA). These trade-offs would be less, or not present, in Johnsongrass. Finally, we also expected Johnsongrass to (iii) grow faster at higher nutrient availability, due to its higher nutrient use efficiency compared to natives.

MATERIALS AND METHODS

Study species and growing conditions

We conducted a greenhouse experiment to evaluate RGR and a set of leaf and resource allocation traits under low and high nutrient availability during the first 7 weeks of growth. The greenhouse experiment was conducted at the USDA-ARS Grassland, Water and Soil Laboratory, Temple, TX, USA. Johnsongrass, switchgrass, little bluestem and big bluestem were grown in monoculture from seeds obtained from a regional commercial seed source (Native American Seed, Junction, TX, USA). We germinated seeds in petri dishes and transferred two seedlings into each cell in propagation trays at the one-leaf stage and then into individual pots at the four-leaf stage, to synchronize seedling development at the beginning of the experiment. Pots were 50 cm tall, 10 cm in diameter and contained a 30/70 sand to topsoil mix. Slow release fertilizer (Osmocote ®; 14-14-14 nitrogen, phosphate and potassium [NPK]) was mixed into the top 5 cm of the soil at 3 g per pot for high NPK or 1 g per pot for low NPK. Plants were transplanted to the pots on September 17th 2012, and were well watered daily until the last harvest on November 1st, when size differences between Johnsongrass and the natives were already well established. Seedlings grew under natural light in a greenhouse. Greenhouse temperature was kept near ambient conditions with evaporative cooling pads.

Pots were arranged in a randomized complete block design, with each of 12 blocks containing one species by fertilization level combination (4 Species *2 NPK *12 Blocks = 96). Blocks were positioned in the greenhouse at a range of distances to the evaporative cooler. We measured leaf photosynthetic rate, root and shoot biomass, leaf area and the nitrogen content of leaves, at days 17, 31 and 45 days post-transplant, on four randomly chosen blocks per date. Day 17 was selected as the earliest harvest date because plants were only then large enough to measure leaf photosynthesis.

Physiological traits

To quantify species physiological traits, we measured the diurnal pattern, light response and CO_2 response of leaf-level photosynthesis. Photosynthesis was measured in the newest fully expanded leaf using a photosynthesis system (LI-6400XT, Li-Cor, Inc., Lincoln, NE, USA) equipped with a 3 × 2 cm leaf chamber and an 85:15 red:blue led light source (6400-02B LED Light Source, Li-Cor, Inc., Lincoln, NE, USA). The leaf area enclosed in the chamber was determined by measuring the width and length of the enclosed leaf blade. Diurnal measurements were conducted at 2.5 h intervals from 8:30 to 16:30. Chamber CO_2 concentration was controlled at 400 ppm, to match ambient conditions in the greenhouse. Cuvette photosynthetic photon flux density (PPFD) was controlled to match greenhouse light for the date and time of day (between 230 and 1500 µmol m^{-2} s^{-1}, depending on the time). Leaf temperature and humidity were also controlled to track ambient conditions (24–40°C; 40–60% RH).

Morphological traits

Aboveground and belowground biomass, leaf area and leaf N percentage were measured at all harvests. Seeds were weighed to represent plant biomass at the beginning of the experiment. We destructively harvested seedlings and separated leaf blade, leaf sheath, stem and root fractions. The leaf blade area was measured with a portable leaf area meter (LI-3000A; Li-Cor, Lincoln, NE, USA). Roots were immediately frozen, and later thawed, washed free of soil and dried. Biomass fractions were determined after drying at 60°C to constant weight for 48 h. Dried blades and sheaths were combined, ground to a fine powder using a ball mill (SamplePrep 8000D, SPEX SamplePrep LLC, Metuchen, NJ, USA), and analyzed for C and N concentrations (%) using a combustion elemental analyzer (Flash 2000 Organic Elemental NC Analyzer, Waltham, MA, USA). Tissue amounts were insufficient for analysis in 9 leaf samples from native species across the experimental design.

Data analyses

We calculated integrated daily rates of carbon (C) uptake per unit leaf area (A′, mol m^{-2} d^{-1}) from the sum of the product of A_{CO2} and the time interval between measurements. Whole-plant daily C uptake was obtained by multiplying A′ by the total leaf area of the plant. SLA (cm^2 g^{-1}) was calculated from the ratio of leaf area to leaf blade dry weight. The leaf-area specific nitrogen mass (N_l; mmol [N] m^{-2}) was calculated from the ratio of leaf N concentration to the product of SLA and the atomic mass of N (g mol^{-1}). Photosynthetic nitrogen-use efficiency (PNUE; mmol CO_2 mol^{-1} N s^{-1}) was calculated from the ratio of A_{CO2} to N_l. The RGR for each species by NPK combination was calculated as the difference between mean natural logarithm-transformed plant weights between harvest dates (Evans 1972, Hoffmann and Poorter 2002).

Statistical analyses were conducted in R version 3.2.2 (R Core Team 2015). To test for differences in total biomass among nutrient treatments and harvest dates, we fit a linear mixed model (package ‘lmerTest’, Kuznetsova *et al.* 2015) with species and fertilizer as fixed effects, harvest date as a covariate and block as a random effect:

$$\begin{aligned}\log\left(\text{total biomass}\right)_{ijkl} = {} & \text{intercept} + \text{species}_i + \text{NPK}_j + \text{harvest}_k \\ & + \text{species}\times\text{NPK}_{ij} + \text{species}\times\text{harvest}_{ik} \\ & + \text{NPK}\times\text{harvest}_{jk} + \text{species}\times\text{harvest} \\ & \times\text{NPK}_{ijk} + \text{block}_l + e_{ijkl}\end{aligned}$$

where subscripts *ijkl* refer to the levels of each treatment and their interactions.

Differences among species growth rates were quantified by regression coefficients computed for each combination of harvest by species by NPK. The significance of differences in biomass among species within harvest dates were tested using post-hoc contrast analyses (F test, package ‘phia’, De Rosario-Martinez *et al.* 2015).

To test whether Johnsongrass and natives differed in the development of plant traits as plant biomass increased and whether differences between Johnsongrass and natives were influenced by nutrient availability, we fitted a linear mixed model with each trait as the dependent variable, and with species and fertilizer as fixed effects. We also included total biomass as a covariate to control for effects that were purely due to size differences (Coleman *et al.* 1994):

$$\begin{aligned}y_{ijkl} = {} & \text{intercept} + \text{species}_i + \text{NPK}_j + \log\left(\text{total biomass}\right)_k \\ & + \text{species}\times\text{NPK}_{ij} + \textit{species}\times\log\left(\text{total biomass}\right)_i \\ & + \text{NPK}\times\log\left(\text{total biomass}\right)_{jk} + \text{species}\times\text{NPK} \\ & \times\log\left(\text{total biomass}\right)_{ijk} + \text{block}_l + e_{ijkl}\end{aligned}$$

where y_{ijkl} represents the response variable, A′, % leaf N, root:shoot ratio, SLA, leaf area, PNUE, or whole-plant daily CO_2 uptake.

When the variance increased with the mean, we used a VarIdent constant variance structure in the mixed model analyses to correct for variance heterogeneity (Zuur *et al.* 2009). The Akaike’s Information Criterion (AIC) of the model was lower when the VarIdent variance term was included than not included ($P < 0.005$). Post hoc analyses of significant main and interaction effects were used to investigate the differences between species in trait development under high or low nutrient levels (package ‘phia’, De Rosario-Martinez *et al.* 2015). When the term including the covariate log (total biomass) was significant, we calculated differences between slope coefficients for each species and NPK level combinations (F test, package ‘phia’, De Rosario-Martinez *et al.* 2015).

Structural equation model

We used structural equation modeling (SEMs, Grace 2006) to test our prediction of different trait trade-offs associated with RGR. SEM identifies which traits are directly and indirectly associated with RGR by considering the correlations between traits and RGR as well as among traits. Our approach was to fit an *a priori* path model across all species, fertilizer levels and harvest dates (Proc Calis, SAS 9.3, SAS Institute, Cary, NC, USA) and then to apply the model to Johnsongrass separately and to the native species combined. Bivariate analyses suggested that plant trait–plant size relationships were often more similar among the native species than between the natives and Johnsongrass. Pooling the natives thus allowed us to generalize trait relationships to RGR in these natives that differed from those of Johnsongrass.

The *a priori* model initially included all variables correlated with biomass increase: root:shoot ratio, total leaf area, SLA, A′, % leaf N, PNUE and whole-plant daily CO_2 uptake. We added or deleted paths when modification indices indicated model fit or parsimony was improved (Buse 1982). The final *a priori* model included SLA, root:shoot ratio, % N concentration and A′ (online supplementary Fig. S1). Several criteria suggested adequate fit of the *a priori* model (X^2 $P = 0.72$; RMSEA < 0.05; probability of close fit >0.05; BBNI >0.90, online supplementary Table S1). Second, the *a priori* model was fit separately to Johnsongrass and the natives to examine if trait relationships to RGR differed between them. We used standardized coefficients to quantify the trait relationships.

RESULTS

Growth analysis

On average, across all harvest dates and fertilizer treatments, biomass was 4-fold greater for Johnsongrass than for the native species (Fig. 1, Species effect $P = 0.06$), despite the fact that seed mass did not differ significantly among species ($P = 0.09$). Plants across species grew faster at high NPK than at low NPK (NPK × Harvest $P < 0.0001$, Table 1). Johnsongrass plants grew the fastest of the four species (Harvest × Species $P = 0.04$, Table 1), achieving a higher RGR during days 0–17 (0.086 ± 0.008 d^{-1}) compared to the natives (0.069 ± 0.005 d^{-1}, post hoc comparison $P = 0.08$). Thereafter, RGRs were not significantly different among species (0.052 ± 0.017 d^{-1} for Johnsongrass and 0.037 ± 0.010 d^{-1} for the native species, post-hoc comparison $P = 0.34$).

Trait analysis

Treatment and species effects on trait values were analyzed with log (total biomass) as a covariate, as traits typically scale allometrically and species could differ in allometric scaling (Table 2). For all species, % leaf N, leaf area and whole plant C uptake increased and PNUE decreased with log (total biomass) across NPK levels ($P < 0.0001$, Figs 2 and 3). The % leaf N increased with log (total biomass) more in big bluestem and little bluestem than in switchgrass or Johnsongrass at low NPK, while at high NPK log (total biomass) increased more for little bluestem and switchgrass (Species × NPK × log (total biomass) $P = 0.03$, Fig. 2c, d and j). In contrast, leaf area and whole plant C uptake increased with biomass more in Johnsongrass than in the native species (Species × log (total biomass) $P < 0.0001$), and Johnsongrass gain in leaf area and whole plant C uptake with plant biomass was amplified at high NPK compared to low NPK (Species × NPK × log (total biomass) $P < 0.0001$, Fig. 3a, b, e and f). SLA was 60% higher in Johnsongrass than natives ($P < 0.0001$), but SLA decreased significantly with log (total biomass) in Johnsongrass, which was not the case for the native species (Species × log (total biomass) $P < 0.0001$, Fig. 2g, h and l). Thus, as plants grew larger, Johnsongrass SLA tended to converge with that of the native species. PNUE was twice as high in Johnsongrass

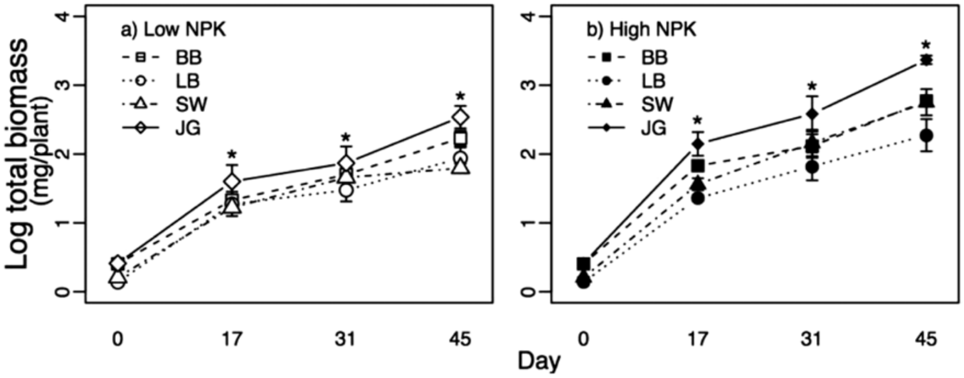

Figure 1: seasonal patterns of total biomass production for big bluestem (BB, *Andropogon gerardii*), little bluestem (LB, *Schizachyrium scoparium*), switchgrass (SW, *Panicum virgatum*) and Johnsongrass (JG, *Sorghum halepense*) growing at low (open symbols, **a**) or high (filled symbols, **b**) NPK levels. Each symbol represents the log transformed mean for the species ± 1 standard error ($n = 4$). The x axis represents the number of days after the plants were transplanted to the pots, where zero = seed mass. Asterisks denote significant differences ($P < 0.05$) between Johnsongrass and the native species within each sampling date.

Table 1: ANOVA results on biomass (log transformed) across harvests, including initial seed mass at day 0

	Log (biomass)	
Effects	nDf,dDF*	*P* value
Harvest date	1, 13	**<0.0001**
Species	3, 91	0.06
NPK	1, 91	0.51
Species *NPK	3, 91	0.88
Species *Harvest	3, 91	**0.04**
NPK*Harvest	1, 91	**<0.0001**
Species *NPK*Harvest	3, 91	0.41

nDf is the numerator degrees of freedom, dDF is the denominator degrees of freedom, following the Satterthwaite approximation (Kuznetsova *et al.* 2015). Significant effects are highlighted in bold.

compared to natives across NPK levels (Species $P = 0.01$), and was lower at high NPK compared to low NPK across species (NPK $P < 0.01$, Fig. 3c and d). Root:shoot ratio and A′ did not vary with log (total biomass) or by species or NPK level ($0.09 \leq P \leq 0.85$, Fig. 2a, b, e and f).

Structural equation model and differences in RGR components between Johnsongrass and natives

The *a priori* SEM identified four traits that separately affected RGR, directly and indirectly across all species: SLA, % leaf N, root:shoot ratio and A′ (online supplementary Fig. S1). When fit separately to Johnsongrass and the native species, the SEM identified several differences in the associations of these traits with RGR (Fig. 4). In the native species, RGR was positively associated with SLA (0.45, $P < 0.0001$) and A′ (0.32, $P < 0.01$), and negatively associated with root:shoot ratio (−0.09, $P < 0.0001$), suggesting that thinner leaves, higher leaf photosynthetic rates and greater shoot allocation all promoted RGR. Concurrently, root:shoot ratio was negatively associated with SLA (−0.33, $P < 0.01$), % leaf N (−0.25, $P < 0.04$), and indirectly with A′ (−0.21, $P = 0.01$, Fig. 4). The total positive effects of SLA (0.51, $P < 0.0001$) and A′ (0.32, $P < 0.01$) on RGR were partially offset by the negative total effects of root:shoot ratio on RGR (−0.26, $P < 0.0001$, Table 3). This means that higher root allocation dampened growth also through indirect effects on other traits. Lastly, the total effects of % leaf N were not significant ($P = 0.71$, Table 3).

In Johnsongrass, RGR was positively associated with A′ (total effect = 0.99, $P = 0.0001$), SLA (total effect = 0.29, $P = 0.04$) and root:shoot ratio (total effect = 0.32, $P < 0.05$) (Fig. 4). Among all traits, A′ had the largest association with RGR. In contrast to the pattern found for native species, higher root allocation benefited RGR in Johnsongrass. RGR was negatively associated with % leaf N (direct effect = −0.78, $P < 0.01$) and positively associated with indirect effects of % leaf N mediated through SLA and A′ [(0.41 × 0.39) + (0.89 × 0.99) = 1.04], so that the total

Table 2 : ANCOVA results for integrated daily photosynthetic rate per unit area (A′), % leaf N, root:shoot, specific leaf area (SLA), total leaf area, photosynthetic nitrogen use efficiency (PNUE) and total C uptake with respect to biomass, Species, NPK and all interaction terms

	Species		NPK		Log (biomass)		Species*NPK		Species* Log (biomass)		NPK* Log (biomass)		Species*NPK* Log (biomass)	
Trait	nDf,dDf	*P*	nDf,dDf	*P*	nDf,dDf	*P*	nDf,dDf	*P*	nDf,dDf	*P*	nDf,dDf	*P*	nDf,dDf	*P*
A′	3, 67.46	0.18	1, 68.40	0.48	1, 36.44	0.13	3, 66.59	0.45	3, 68.19	0.13	1, 67.05	0.50	3, 67.05	0.40
% leaf N	3, 60.05	**<0.01**	1, 62.90	**0.01**	1, 47.22	**0.0001**	3, 58.96	**0.02**	3, 60.69	**<0.0001**	1, 60.62	0.47	3, 59.64	**0.03**
Root:Shoot	3, 67.09	0.34	1, 67.98	0.85	1, 30.99	0.17	3, 66.37	0.35	3, 67.77	0.09	1, 66.97	0.21	3, 66.88	0.56
SLA	3, 64.80	**<0.0001**	1, 65.38	0.16	1, 77.81	0.29	3, 64.56	0.77	3, 65.06	**<0.0001**	1, 64.59	0.70	3, 64.63	0.82
Leaf area	3, 72.56	**<0.0001**	1, 73.13	**<0.0001**	1, 25.22	**<0.0001**	3, 71.63	**<0.0001**	3, 73.42	**<0.0001**	1, 72.58	**<0.0001**	3, 72.44	**<0.0001**
PNUE	3, 65.43	**0.01**	1, 64.75	**<0.01**	1, 29.81	**<0.0001**	3, 63.94	0.19	3, 66.30	0.27	1, 64.62	0.059	3, 65.46	0.15
Total C uptake	3, 69.73	**<0.0001**	1, 70.22	**<0.0001**	1, 44.08	**<0.0001**	3, 69.28	**<0.0001**	3, 70.27	**<0.0001**	1, 69.20	**<0.0001**	3, 69.62	**<0.0001**

Significant effects are highlighted in bold lettering. Shown are the *P* values, nDf (numerator degrees of freedom) and dDF (denominator degrees of freedom) using the Satterthwaite approximation (Kuznetsova *et al.* 2015).

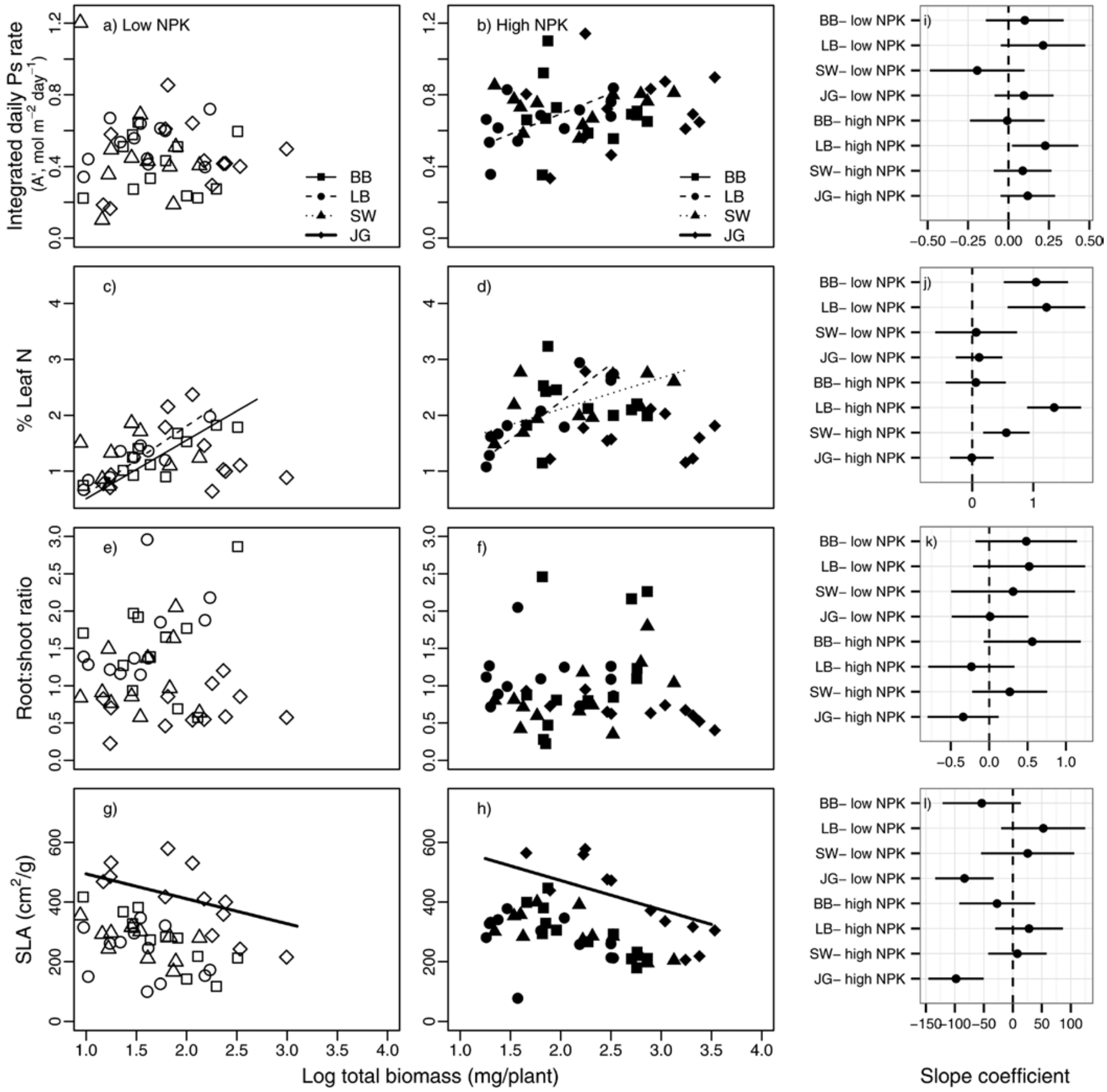

Figure 2: change in morphological and physiological traits with changes in plant biomass (log transformed) across plant development (measurements taken at 17, 31 and 45 days after transplant) for big bluestem (BB, *Andropogon gerardii*), little bluestem (LB, *Schizachyrium scoparium*), switchgrass (SW, *Panicum virgatum*) and Johnsongrass (JG, *Sorghum halepense*) growing at low (open symbols) or high (filled symbols) NPK levels. Each symbol represents biomass (log transformed) and trait values for each plant (n = 96). Linear models relating log biomass and traits are shown as bold solid lines for Johnsongrass and thin lines for natives when significant at $P < 0.05$. Right panel: Solid lines represent 95% confidence intervals for estimated effect of log (biomass) on plant traits for each species by NPK combination. Dots represent point estimate of slope values. See Table 2 for significance of fixed effects and covariate.

effect of %leaf N on Johnsongrass RGR was not significant (P = 0.18, Table 3).

DISCUSSION

In this study, we aimed to identify the trait combinations that give Johnsongrass a growth advantage in the early stages of seedling growth. First, we did confirm that Johnsongrass seedlings grew faster between emergence and day 17, which lead up to a 4-fold size advantage by the end of the experiment. Johnsongrass differed from the native species in several traits, including higher SLA and PNUE, greater leaf area, and lower % leaf N and allocation to roots. The structural equation model indicated that RGR in Johnsongrass was most strongly and positively associated with carbon gain and SLA, and a positive effect of root biomass increase on RGR (Fig. 4). In contrast, the native species exhibited a negative effect of allocation to roots, which made RGR comparatively less dependent on A′ and more constrained by SLA. Thus, when establishing from seed, which may be crucial in the first stages of invasion, the competitive strategy and invasion success of Johnsongrass can be explained at least partially by its aggressive investment in acquiring C.

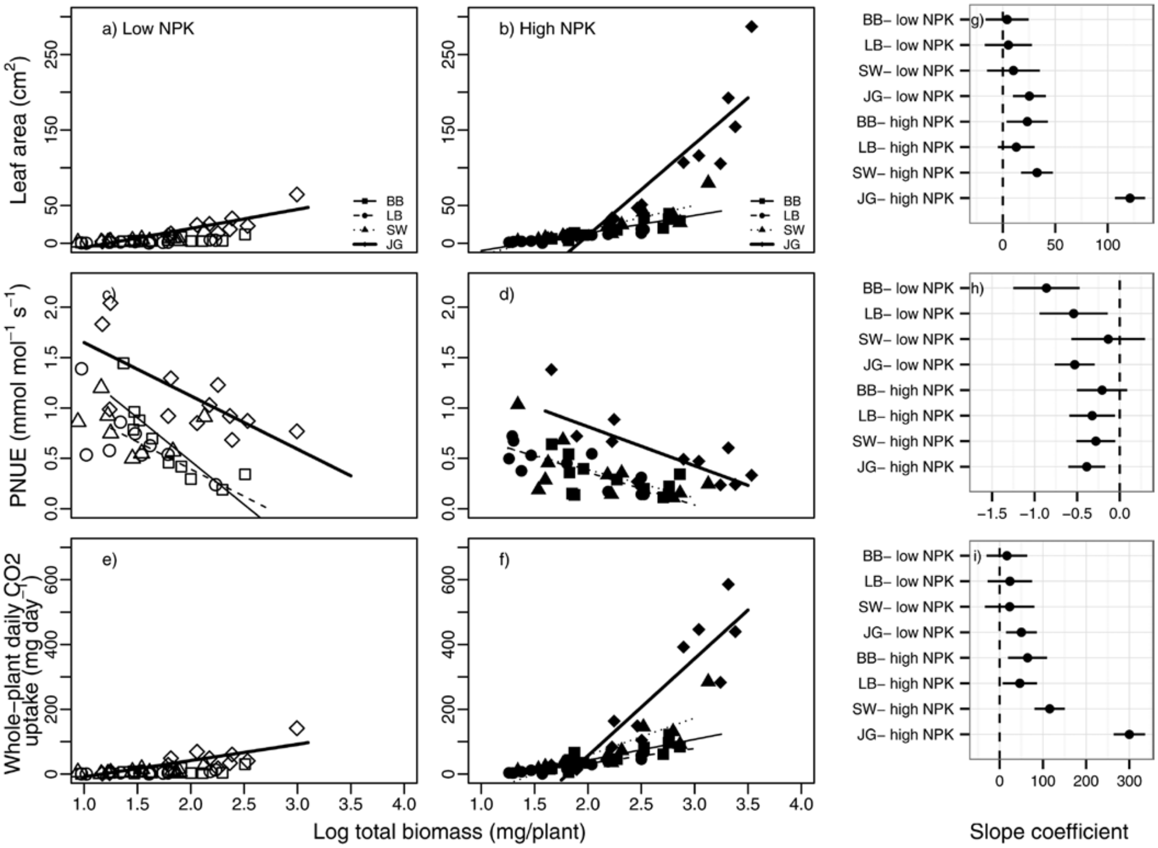

Figure 3: change in leaf area, PNUE and whole-plant daily CO_2 uptake with changes in plant biomass (log transformed) across plant development (measurements taken at 17, 31 and 45 days after transplant) for big bluestem (BB, *Andropogon gerardii*), little bluestem (LB, *Schizachyrium scoparium*), switchgrass (SW, *Panicum virgatum*) and Johnsongrass (**JG**, *Sorghum halepense*) growing at low (open symbols) or high (filled symbols) NPK levels. Each symbol represents biomass and trait values for each plant (n = 96). Filled lines correspond to the linear model relating log biomass and trait for Johnsongrass, dashed lines correspond to natives linear model between log biomass and trait. Right panel: Solid lines represent 95% confidence intervals for estimated effect of log (biomass) on plant traits for each species by NPK combination. Dots represent point estimate of slope values. See Table 2 for significance of fixed effects and covariate.

While invasive plants often have higher SLA and shoot allocation than long-lived perennial species of the same plant functional type (Allred *et al.* 2010; Dohleman *et al.* 2009; Smith and Knapp 2001; van Kleunen *et al.* 2010), few studies have examined how these traits translate into a net-positive effect on growth, given that more leaves and faster growth also produce a greater demand for nutrient uptake, which, if not met, could reduce rates of leaf photosynthesis. For Johnsongrass in our study, the crucial factor was that seedlings could maintain the same rate of photosynthesis with lower root:shoot ratio and % leaf N than natives, due to its high PNUE (Fig. 2). However, this strategy paid off only in the early phases of growth, since RGRs were not different among species after day 17. This dampening of the growth advantage may be explained by the strong positive correlation between % leaf N and SLA. As seedlings grew and leaves became larger and thicker, they needed more structural support and their % leaf N declined. It is possible that this dynamic rapidly constrained Johnsongrass growth compared to the other species.

Unexpectedly, nutrient addition increased biomass of Johnsongrass and the natives to similar extents during the first 45 days of growth. In contrast, species responded differentially to nutrient addition in % leaf N, leaf area and whole plant C uptake (Table 2). Nutrient addition increased Johnsongrass % leaf N less than the native species but increased total leaf area more, suggesting a developmental strategy in Johnsongrass that prioritizes leaf area growth and C gain. Indeed, Johnsongrass plants showed higher whole-plant C uptake than native species at the last harvest. However, this strategy provided no clear advantage in biomass gain to Johnsongrass at high NPK. It is therefore possible that the same constraint that made the growth advantage short-lived, e.g. the need for greater structural support as leaves grew larger, also dampened the effect of higher nutrient levels on growth, because structural support would have been needed earlier in faster-growing plants. This finding supports previous studies showing that Johnsongrass responds similarly to other perennial C_4 grasses, including switchgrass, to elevated nutrient levels (Hoffman and Buhler 2002; Kering *et al.* 2011; McLaughlin *et al.* 2004; Ra *et al.* 2012).

No single trait explained interspecific variation in RGR, a finding which is consistent with other studies showing that

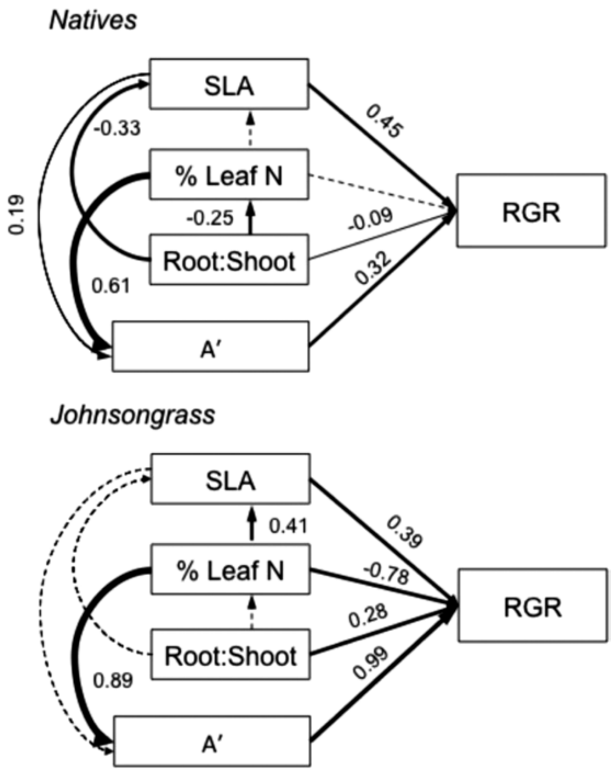

Figure 4: structural equation models for natives and Johnsongrass growing across NPK treatments across dates, showing hypothesized relationships among specific leaf area (SLA), root:shoot, % leaf N and integrated daily photosynthetic rate per unit area (A′). Standardized coefficients are given for each path. Significant paths are indicated by solid arrows ($\alpha \le 0.05$) with arrow thickness correlating with the absolute value of the standardized coefficient; non-significant paths included in the model are shown with dashed arrows.

trait combinations explained species variation in establishment and cover (Roberts *et al.* 2010) or invasion success (Küster *et al.* 2008) better than single traits. Our experiment also suggested a mechanistic basis for the interactive effects of traits on growth, at least for the comparatively simple seedling stage. Every growth strategy is constrained by trade-offs, but trade-offs had different consequences for native and invasive species in terms of species niche opportunities and vulnerabilities. Thus, not only it is critical to examine multiple traits to explain why a species is invasive, but also to understand how multiple traits interact to determine RGR. Differences in the trade-offs between traits that promote rapid growth between natives and Johnsongrass would not be evident by looking just at single traits, or multiple traits in a bivariate framework.

Equally important, trait values change over time. Johnsongrass was most different from the native species 17 days after germination. Afterwards, Johnsongrass and natives converged in distinct traits such as SLA. Looking at trait values as soon as they can be measured on developing seedlings thus proved crucial to understanding invasive success in Johnsongrass. Studies that measure trait values only on plants well advanced in maturity may miss crucial trait differences altogether.

Table 3. Total standardized effects for SEM (see Fig. 4)

	Total standardized trait effects	
	On Natives RGR	On Johnsongrass RGR
SLA	0.51 ± 0.04; ***P*** < **0.0001**	0.29 ± 0.14; ***P*** = **0.04**
A′	0.32 ± 0.12; ***P*** < **0.01**	0.99 ± 0.26; ***P*** = **0.0001**
Root:shoot	−0.26 ± 0.06; ***P* < 0.0001**	0.32 ± 0.16; ***P*** < **0.05**
% leaf N	0.05 ± 0.12; *P* = 0.71	0.23 ± 0.17; *P* = 0.18
	on Natives SLA	on Johnsongrass SLA
Root:shoot	−0.29 ± 0.11; ***P* = 0.01**	0.11 ± 0.20; I = 0.59
% leaf N	−0.15 ± 0.12; *P* = 0.22	0.41 ± 0.17; ***P*** = **0.02**
	on Natives A′	on Johnsongrass A′
SLA	0.18 ± 0.09; *P* = 0.06	−0.10 ± 0.12; *P* = 0.39
Root:shoot	−0.21 ± 0.08; ***P*** < **0.01**	−0.002 ± 0.18; *p* = 0.99
% leaf N	0.58 ± 0.08; ***P*** < **0.0001**	0.84 ± 0.06; ***P*** < **0.0001**

Total effects represent the sum of direct and indirect effects of physiological and morphological traits on relative growth rate (RGR), specific leaf area (SLA) and integrated daily photosynthetic rate per unit area (A′) for natives (big bluestem, little bluestem and switchgrass) and Johnsongrass.

CONCLUSIONS

Johnsongrass grows more rapidly than three other functionally similar native C_4 grasses during the earliest days of seedling growth. The basis for faster early seedling growth in Johnsongrass appears to lie in its investment in carbon gain. Moreover, higher nutrient levels did not cause an even greater growth advantage probably because of a negative feedback between plant biomass and the need for structural support tissues, which reduced SLA and thus RGR with the increasing biomass of seedlings. For our sample of three functionall similar native grasses, the investment in carbon gain was constrained by the investment in roots, likely for acquiring nutrients. It seems that the combinations of traits evident in the earliest phases of growth form the basis for a competitive advantage for Johnsongrass growing among functionally similar native grasses.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

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