

Short-term effects of elevated precipitation and nitrogen on soil fertility and plant growth in a Neotropical savanna

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Abstract. Increasing nitrogen (N) deposition and changing precipitation patterns in Neotropical savannas could alter plant growth, reproduction, and nutrients by altering soil nutrient and water availability. We examined the potential for simulated N deposition and increased dry season precipitation to have interactive effects on reproduction and growth of two abundant native Cerrado (Brazilian savanna) grasses—*Loudetiopsis chrysothrix* and *Tristachya leiostachya*—via feedbacks with soil nutrient status. Plant growth and reproduction responses consistently varied by species. Water addition led to more consistent increases in both growth and reproduction than nitrogen addition and the two treatments did have significant interactive effects. We expected that both treatments would affect plant growth and reproduction via positive effects on soil and plant N. Instead, we found that plant responses were linked to species-specific treatment effects on soil and foliar phosphorus (P). Structural equation models (SEM) confirmed that changes in soil P—rather than changes in soil N or increasing soil acidity—explained plant response to treatments. Our results imply that N deposition and precipitation change could impact Cerrado plant growth and reproduction via subtle effects on plant and soil phosphorus.

Key words: Brazil; bunchgrass; Cerrado; *Loudetiopsis chrysothrix*; nitrogen enrichment; phosphorus; precipitation change; savanna; soil fertility; *Tristachya leiostachya*.

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INTRODUCTION

Anthropogenic nitrogen (N) addition has more than doubled pre-industrial nitrogen inputs to terrestrial environments (reviewed in Schlesinger 2009). Increased N deposition changes soil acidity and macronutrient availability (Vitousek

et al. 1997, Aber et al. 1998) which alters plant biomass allocation, phenology, fitness, and competitive interactions (Cleland et al. 2006, Clark and Tilman 2008, Lau et al. 2008). The effects of N deposition on plant growth and soil fertility can affect ecosystem net primary production, plant diversity, and global climate via effects on carbon

cycling (Vitousek et al. 1997, Gruber 2008). However, the direction and magnitude of the plant responses to N deposition can vary widely due to the nutrient requirements of different plant functional groups and species (Craine et al. 2002, Zavaleta et al. 2003) as well as the limitations imposed by soil fertility and regional climate (Bobbink et al. 2010).

In addition to increasing nitrogen deposition, human-caused increases in atmospheric CO₂ are altering the timing and abundance of precipitation world-wide (Zhang et al. 2007). Plant phenology and growth can be highly sensitive to changes in precipitation (Fay et al. 2003, Zavaleta et al. 2003, Kochy and Wilson 2004), particularly in ecosystems with long dry seasons where nitrogen mineralization and plant nitrogen uptake are synchronized with seasonal precipitation patterns (Austin et al. 2004, Knapp et al. 2006, Yahdjian et al. 2006). Results from N deposition and precipitation experiments suggest that these global change factors can have unpredictable and interactive effects on plant flowering and growth (Zavaleta et al. 2003, Cleland et al. 2006, Henry et al. 2006, Siemann et al. 2007).

Because many tropical savannas and grasslands experience profound dry-seasons, they may be particularly sensitive to changes in the timing (Knapp et al. 2002) or absolute amount (Pandey and Singh 1992, Kochy and Wilson 2004) of precipitation. Tropical savannas may also be highly sensitive to nitrogen deposition because plant productivity tends to be primarily limited or co-limited by N (Barger et al. 2002, Augustine 2003, Sarmiento et al. 2006). In N-limited grasslands and savannas, plant response to precipitation amount and timing is linked to the effects of rainfall on seasonal N mineralization patterns (Seagle and McNaughton 1993, Austin et al. 2004, Yahdjian et al. 2006) and mass-flow of inorganic N to plant roots (Borke and Matzner 2009). Despite projections of sharp increases in N deposition (Phoenix et al. 2006) and predictions for precipitation change (Christensen et al. 2007) in tropical savannas, few experiments have explicitly tested how native plants and soils are likely to respond to these global changes (reviewed by Matson et al. 1999, Bobbink et al. 2010). The unique characteristics of tropical savannas could lead to responses to

global changes that diverge from those observed in the extensive experimental results from temperate grasslands (e.g., Fisher and Whitford 1995, Carrera et al. 2003, Zavaleta et al. 2003, Kochy and Wilson 2004, Cleland et al. 2006).

Among tropical savannas, the Neotropical Brazilian Cerrado is remarkable for its biodiversity (10,000 plant species) and extent (2 million km²) (Oliveira and Marquis 2002). Cerrado ecosystem function and diversity is threatened by land-use change, invasive grasses, and urbanization (Ratter et al. 1997). In addition, increases in urban fossil fuel combustion and N fertilizer use are expected to more than double N deposition rates in the Cerrado, from an average of 5–13 kg N ha⁻¹ yr⁻¹ in the 1990s to 14–38 kg N ha⁻¹ yr⁻¹ by 2050 (Bustamante et al. 2006, Phoenix et al. 2006). N deposition rates of this magnitude are associated with plant species loss and decreased productivity in North America (Clark and Tilman 2008) and Europe (Stevens et al. 2004) and have the potential for similar negative impacts on Cerrado species and ecosystem processes (reviewed in Bobbink et al. 2010).

Nitrogen deposition could decrease soil fertility and plant growth in the Cerrado and other nutrient poor tropical ecosystems by decreasing cation and phosphorus availability, increasing acidity, and raising levels of toxic aluminum (Matson et al. 1999). However, the potential for negative effects may depend upon plant and soil demand for added N given extremely nutrient poor Cerrado soils. N deposition is more likely to have negative effects on Cerrado plant species if P is the co-limiting or primary nutrient limiting growth because P is likely to be reduced with increased soil acidity. P-limitation in Cerrado ecosystems has been suggested by some previous experiments and observational studies (Bustamante et al. 2006, Nardoto et al. 2006, Kozovits et al. 2007). However, there is also evidence for N limiting or co-limiting plant productivity—as is often the case in temperate savannas and grasslands (Bustamante et al. 2006). Conflicting evidence for nutrient limitation in the Cerrado may result from the apparent wider range of nutrient use efficiencies among Neotropical savanna and rainforest species compared to temperate species (Bustamante et al. 2004, Townsend et al. 2007).

Here we report the results of a year-long

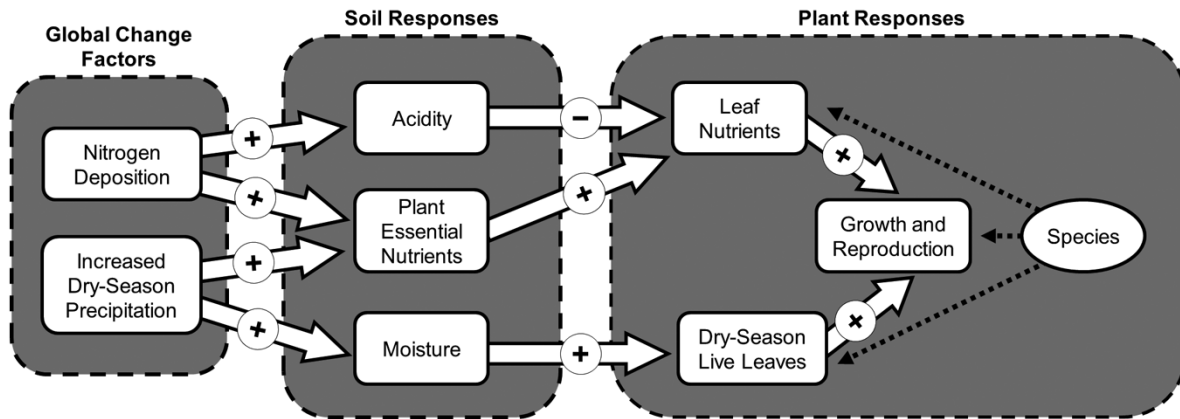


Fig. 1. Conceptual framework for potential positive and negative effects of N deposition and dry-season precipitation increase on plants via effects on soil fertility.

experiment testing the interactive effects of N deposition and precipitation change on plant biomass allocation and soils in the Cerrado with two dominant C-4 native grass species, focusing on the links between soil and plant responses (Fig. 1). We experimentally added nitrogen in amounts and rates consistent with nitrogen deposition levels for the region for the coming century (Phoenix et al. 2006). We based our water addition treatment on climate change models which predict increasing dry season precipitation in the Cerrado under moderate climate change scenarios, though some models and climate change scenarios also predict decreases in precipitation (Magrin et al. 2007).

Our focal species were chosen based on their co-dominance in our study system, suggesting that any species-specific responses to global change factors could have important implications for plant and soil properties ecosystem-wide. For example, increased growth of the dominant grass species could negatively affect woody plant recruitment and affect the relative abundance of woody plants and grasses, a key element of savanna structure and ecosystem dynamics (Gardner 2006, Furley 2007). In addition, studies with co-dominant C-4 grass species in North American prairies (Silletti and Knapp 2001, Swemmer et al. 2006, Nippert et al. 2009) have demonstrated that co-dominant species may respond differentially to global changes due to trade-offs in resource use and acquisition strategies.

Our research addressed two central questions for predicting the effects of the combination of N

deposition and precipitation change on Cerrado ecosystems. First, we asked: How do N addition and increased dry season water availability affect the nutrient status of infertile Cerrado soils? We expected that both treatments could increase soil N, either directly with N addition, or indirectly with water addition via the positive effects of soil moisture on plant-available N. In contrast, we expected that N addition could have a negative effect on soil fertility if N addition increased soil acidity, which could further decrease the availability of P and essential cations in acidic, nutrient-poor Cerrado soils. Second, we addressed the question: How do N and water addition affect growth and reproduction of co-dominant grass species? We expected that both treatments would lead to increased growth and reproduction because N and dry-season water are potentially limiting in this ecosystem.

Finally, our approach allowed us to evaluate whether plant responses to N-deposition and dry season water addition were due to the positive (fertilizing) or negative (toxicity) plant-soil feedbacks. Specifically, we expected that treatment effects on plant growth and reproduction would be linked to changes in changes in soil essential nutrients, pH, and toxic aluminum levels via increases or decreases in leaf senescence, foliar nutrients, and root:shoot ratio (Fig. 1). We constructed structural equation models to test for support of positive vs. negative plant-soil feedbacks and to compare the relative importance of direct versus indirect (soil-mediated) effects of N-deposition and water addition on plant responses.

MATERIALS AND METHODS

Study site and species

This study was conducted at the Estação Ecológica do Panga, a 404 ha reserve located 40 km from Uberlândia, Minas Gerais, Brazil (19°10' S, 48°23' W). Monthly average temperatures range from 20–25°C, and annual rainfall is approximately 1600 mm with an almost rainless dry season from May and September (Instituto de Geografia 2008). Soils are highly weathered Oxisols with a high clay content and low pH (Latossolo Vermelho-Amarelo, Brazilian soil taxonomy EMPRAPA 1999, Anionic Acrustoxe, US soil taxonomy, Soil Survey Staff 2003). Our study was conducted in *cerrado ralo*, a vegetation physiognomy typified by dense grass cover interspersed with small trees and shrubs (Cardoso et al. 2009). The preserve is protected from grazing and other agricultural activities, but is subject to occasional anthropogenic fires originating on adjoining roads. The most recent fire in the study area occurred 2 years before the start of our experiment.

Our focal species were two native C-4 perennial bunchgrasses in the Tribe Arundinellae, *Tristachya leiostachya* and *Loudetiopsis chrysothrix* (referred to hereafter by genus). *Tristachya* is generally larger than *Loudetiopsis*: the average *Tristachya* genet (individual bunchgrass) is 25 cm in diameter, whereas *Loudetiopsis* genets are 10 cm in average diameter. *Tristachya* vegetative tillers are 90 cm tall on average and *Loudetiopsis* vegetative tillers are 70 cm tall on average. Together they account for 69% of the above-ground biomass in the study area (*Loudetiopsis* = 12%, *Tristachya* = 57%, all other species < 5% each, E. M. Bruna and H. L. Vasconcelos, unpublished data), with peak biomass and flowering occurring between February and April. Both species also have broad distributions: *Tristachya* ranges from southern Brazil to Paraguay and *Loudetiopsis* from eastern Bolivia to southern Brazil and Paraguay (Missouri Botanical Garden 2009).

Experimental design

In May 2008 we randomly selected N = 80 individual bunchgrasses of each species in a 150 × 200 m area of homogenous aspect, slope, and vegetation cover. The plants were located along 6

transects 50 m apart; transects were 150 m (5 transects) or 50 m (1 transect) long. Individuals were within 1/3 of the median diameter for individuals of that species (*Tristachya*: 18.3–36.7 cm, *Loudetiopsis* 9–18 cm, based on a random sample of N = 15 individuals in the study area) and all plants used in the experiment were at least 2 m from any other focal plants. We established plots of 50 × 50 cm around each selected individual and randomly assigned N = 20 individuals of each species to one of four treatments: nitrogen addition, water addition, nitrogen addition × water addition, and unmanipulated controls. We reduced the potentially confounding effects of aboveground competition by clipping all above-ground biomass surrounding the focal individual before the treatments and throughout the experiment at 2–3 week intervals. All below-ground biomass and leaf litter were left undisturbed to limit effects on decomposition and roots.

Treatments

We added a total of 25 kg ha⁻¹ yr⁻¹ N (2.5 g m⁻¹ yr⁻¹ N) to plants in the N treatment in accordance with predicted N deposition levels for the region (approximately 12 kg ha⁻¹ yr⁻¹ N, with a maximum of approximately 38 kg ha⁻¹ yr⁻¹ N by 2050; Phoenix et al. 2006). The nitrogen was added as ammonium nitrate (NH₄NO₃) in four applications of slow-release commercial fertilizer (31% N; Manah, Gunge Fertilizantes S/A, Uberaba, MG, Brazil) sprinkled evenly on soil surface in the plot (June, September, and December 2008, February 2009). Ammonium nitrate (50% each ion) is a reasonable approximation of N deposition for this site: from 1997–1999 N wet deposition near Uberlândia was 48% ammonium and 38% nitrate (Lilienfein and Wilcke 2004).

Climate models for the Cerrado region predict both increases and decreases in rainfall by 2099 under modest climate change scenarios (Christensen et al. 2007). We chose to experimentally add precipitation in our experiments based on increasing regional precipitation trends over the last 40 years (Haylock et al. 2006). We added water to plants in the precipitation addition treatment with drip irrigation at a rate of two liters per 24 hours (8 mm/day) in the middle of the dry season of 2008 (June–August); each treatment was divided by alternate dry periods

of 2 and 9 days. In total we added 72 mm of water, approximately 5% of the average annual rainfall or seven times the ambient 2008 dry season precipitation (9.5 mm from June–August 2008, data from Uberlândia, 40 km from site, 2003–2008, Instituto de Geografia 2008). Our daily water addition rate was a substantial increase over average monthly dry season precipitation rates (11.0 ± 13.9 mm rainfall/month [mean \pm SD] from June–August 2004–2008) and was comparable to half the average daily precipitation rate during the wet season (October 2003–April 2004, 13.3 ± 4.0 mm/day). As a result, average daily volumetric water content (m^3/m^3) in watered plots was approximately three times greater than in un-watered plots (season mean = 0.03, lower 95% CL 0.02, upper 95% CL 0.04 vs. season mean = 0.01, lower 95% CL 0.01, upper 95% CL 0.02, respectively) and average, maximum, and minimum daily soil moisture were significantly different ($p < 0.01$) between watered and un-watered plots during the water addition period (see Appendix).

Light availability

Because, light limitation can decrease N limitation and shady microclimates tend to ameliorate water limitation in tropical savannas (Cruz 1997, Ludwig et al. 2001), we quantified shading for each individual by measuring photosynthetically active radiation (PAR) in our plots with an 80 cm long quantum line sensor ceptometer (Accupar LP80, Decagon Devices, Pullman WA) and incorporated relative light availability as a covariate in our analyses. PAR was quantified between 11 am and 2 pm on March 3, 2009 and was measured at the height of the tallest leaves of each individual to quantify shading by overstory vegetation (primarily trees and shrubs). We recorded the average of three measurements through the center of the plot taken at different orientations parallel to the ground.

Soil and foliar nutrients

To measure resin-available N (NH_4^+ and NO_3^-), we installed mixed bed resin bags in the top 10 cm of soil surrounding 76 of our experimental plants ($N = 32$ *Loudetiopsis* and $N = 44$ *Tristachya*). Resins were charged with 1 M NaCl and extracted with 2 M KCl after 28 days of

field incubation (February–March 2009). Bulk inorganic N pools were quantified by sampling the upper 10 cm of soil in March 2009 ($N = 50$ *Loudetiopsis* and $N = 49$ *Tristachya*). We measured N availability by extracting 10 g samples of field-moist soil with 2 M KCl and adjusted for soil gravimetric water content and bulk density. Concentrations of NO_3^- and NH_4^+ in soil and resin extracts were analyzed colorimetrically with an Astoria Autoanalyzer (Astoria-Pacific, Clackamas, OR, USA). Both ionic forms were analyzed because of the potential of the treatments to have differential effects on the two soil ions. For example, water addition could increase both microbial mineralization of ammonium and nitrification of nitrate, while the effect of N addition on soil N forms might depend on plant and microbe N demand and uptake.

We quantified the effects of our experimental treatments on indicators of soil acidity and toxicity (pH and Al), phosphorus, and essential cations (K and Ca) using soil samples collected in March 2009 and dried at 55°C for 48 hours ($N = 10$ per treatment \times species combination). We measured pH in deionized water (ratio: 1:2.5 soil : H_2O). Potassium (K) and phosphorous (P) were extracted with Mehlich ($\text{HCl}-\text{H}_2\text{SO}_4$) solution (K ratio: sample : solution 10:1, P sample: solution 20:1). K concentration was determined by flame emission spectrophotometry (Model No. B462, Micronal, São Paulo, SP, Brazil) and P concentration with UV/Visible spectrophotometry (Cary 50 Conc UV-Vis, Varian, Palo Alto, CA, USA). We extracted aluminum (Al) and calcium (Ca) in 1 M KCl (Ca ratio: 100:1, Al ratio: 10:1 sample : solution). Al concentration was determined by titration with NaOH in the presence of bromothymol blue. Ca concentrations were determined with atomic absorption spectrophotometry (Model No. 932 A, GBC Scientific Equipment, Dandenong, VIC, Australia).

We measured the effects of our treatments on foliar N and P by sub-sampling 20 green undamaged leaves from each individual. Change in specific leaf area was not quantified because this metric would have required removing a significant proportion of an individual's green leaves pre-treatment with potential effects on other plant responses to treatments. Leaves were washed with deionized H_2O , dried for 48 hours at 60°C, and ground in a plant mill (Marconi

Equipamentos, MA 048, Piracicaba, SP, Brazil). Foliar N was determined by Kjeldahl digestion, steam distillation of the digest into H_3BO_3 , and titration with H_2SO_4 . We extracted foliar P from ground tissue with HNO_3 and HClO_4 and determined digest P concentration with UV/Visible spectrophotometry (Model No. Cary 50 Conc UV-Vis, Varian, Palo Alto, CA, USA).

Plant reproductive effort, growth, and biomass allocation

Bunchgrasses reproduce in two distinct manners (1) through seedling recruitment and (2) production of lateral tiller (Tomlinson and O'Connor 2004). For our study we quantified reproduction based on measures of reproductive effort and allocation related to seedling recruitment by measuring (1) flowering vs. non-flowering individuals, (2) the number of flowering tillers per individual, (3) the total number of spikelets, and (4) allocation of spikelets per flowering tiller. Each spikelet corresponded to one fertile floret which could potentially have developed into one viable seed and we observed that the flower structures of both species were consistent with sexual reproduction. However, we did not directly measure the number of developed seeds, their viability, or loss due to herbivory. Measurements were made both before and after the treatments were applied (May 2008 and March 2009, respectively).

We measured the diameter around the base of each genet (individual bunchgrass) before and 10 months after the treatments were applied and calculated percent growth because of the effect of size on growth rate. We used bunch diameter to quantify growth because bunchgrass above-ground biomass is tightly correlated with diameter in many bunchgrass species (Nafus et al. 2009) due to bunchgrass growth through tiller production (Tomlinson and O'Connor 2004) allowing diameter to be used as a surrogate measure of growth for grasses with this habit (e.g., Marty et al. 2005). We also did not observe any evidence of rhizomatous growth for any of the individuals we excavated. For our control individuals of our focal species we found that diameter is significantly correlated with total aboveground biomass (*Loudetiopsis*: $R^2 = 0.23$, $p < 0.001$, $N = 79$. *Tristachya*: $R^2 = 0.28$, $p < 0.001$, $N = 80$) and total meristems (*Loudetiopsis*: $R^2 = 0.40$,

$p < 0.001$, $N = 79$. *Tristachya*: $R^2 = 0.45$, $p < 0.001$, $N = 80$).

To estimate root : shoot ratio, we collected all plants at the peak of the growing season (4 weeks March–April 2009, collection date randomized to eliminate systematic effects of collection date on biomass), separated the roots, live leaves, dead leaves, flowering stems, and flowering spikes (i.e., florets and seeds), and dried the material at 55°C until the samples reached constant weight (2–4 days). We recovered as much of the root biomass as possible by trenching around the perimeter of the plot and excavating to below the depth of the main root mass (i.e., approximately 15 cm for *Loudetiopsis* and 20 cm for *Tristachya* individuals). We tested the efficacy of the root collection method by collecting five 6 cm wide \times 5 cm deep cores in the soil remaining in the plot area after plants were removed, sieved for coarse root mass (2 mm sieve), and dried the material to constant weight at 55°C . Root mass remaining in the soil averaged 0.002 ± 0.001 g/cm² (mean \pm SE), while the average root mass of our excavated grasses was 62.33 ± 38.28 g for *Loudetiopsis* and 399.69 ± 255.83 g for *Tristachya*, suggesting that we were able to recover the vast majority of the grass root systems. Finally, we estimated reduction in leaf senescence by counting all green leaves on the plant and dividing the number by genet area in August of 2008 to calculate green leaf density. Higher green leaf density corresponded to more green leaves per plant area (decreased leaf senescence) during the dry season.

Statistical analyses

Treatment effects.—To test for species and treatment effects on soil parameters (bulk soil NH_4^+ , resin available NO_3^- , pH, Mehlich P and K, KCl-extracted Ca and Al) we used general linear models with nitrogen, water, and species as fixed effects. Treatment and species effects resin available NH_4^+ and bulk soil NO_3^- were analyzed with generalized linear models (gamma distribution). All plant response variables were analyzed by species with nitrogen and water as fixed effects. The likelihood that an individual would flower (response: flowering or non-flowering) in response to treatments and separately, to resin available NO_3^- and NH_4^+ , were analyzed with binomial models. To exam-

ine treatment effects on the number of flowering tillers per flowering individual and number of total spikelets we used generalized linear models with negative binomial distributions. General linear models were used to evaluate treatment effects on the number of spikelets per tiller. To test for the effects of our treatments on growth, we used an ANCOVA with the percent difference in diameter between year one and year two as the response variable, nitrogen and water as fixed effects and original diameter and average PAR as covariates. We tested for treatment effects on senescence (number of green leaves by area) with an ANCOVA with original diameter as a covariate. We compared the response of root : shoot ratios to our treatments with a gamma distributed generalized linear model. We analyzed the effects of treatments on total aboveground biomass, total dead leaf biomass, and total live leaf biomass with general linear models. We tested for differences in foliar N and P concentrations and N:P ratios in response to treatments with an ANCOVA with live leaf mass as the covariate to control for the possibility that a change in leaf biomass could have affected nutrient concentration.

Structural equation models.—We used structural equation models (SEMs) to evaluate (1) a priori hypotheses for positive or negative direct or indirect effects (via soils) of treatments on plants (illustrated in Fig. 1) and (2) an a posteriori model for hypothesized relationships between treatments, soils, and plants based on the results of our univariate tests (Grace 2006). Both the a priori and a posteriori tests were “confirmatory” uses of structural equation models. This application of SEMs allowed us to test whether our experimental data confirmed the hypothesized mechanistic relationships between variables in the system (Grace and Pugsek 1998). Structural equation models which fail to fit the data can be rejected based on p-values (e.g., $p < 0.05$) while models which cannot be rejected (e.g., $p > 0.05$) are considered adequate models for the data structure (Grace 2006). Non-significant p-values for variable relationships (paths) in acceptable models do not indicate poor fit of the over-all model (Mitchell 1992). For both types of models, standardized coefficients are calculated to allow for direct comparison of relationships between variables despite differences in measurement

scale (Grace 2006).

For our a priori models we constructed models for our hypothesized relationships between treatment, soil and plant characteristics for negative and positive soil-plant feedbacks (both positive and negative paths illustrated in Fig. 1). If N addition led to negative plant-soil feedbacks we expected that N addition would increase soil acidity, leading to higher levels of toxic Al, decreased P, and ultimately reduced plant growth (diameter change) and reproduction (number of flowering culms). If water and N addition led to positive soil-plant feedbacks we expected that water addition would increase N mineralization (resin NO_3^- and NH_4^+) and reduce leaf senescence while N addition would lead increased available N (resin NO_3^- and NH_4^+) leading to increases in foliar N:P and plant growth and reproduction. We expected that species would strongly affect plant responses given the potential for coexisting species to have divergent resource use and responses to changes in N and water availability. The negative soil-plant feedback model was constructed by omitting the precipitation treatment because water addition was not expected to negatively affect soil fertility and including soil P, Al, and pH as indicators of soil toxicity. The positive soil-plant feedback model included both the water and nitrogen treatments, species, soil N (resin NO_3^- , NH_4^+), foliar N:P, number of flowering culms, percent diameter change, and light (PAR).

We constructed our a posteriori SEM based on the ANOVA results for treatment effects on soil and plant variables. In this model, P was the only soil variable included and both water and nitrogen treatments were incorporated into the model. Model fit and parameter values were calculated with maximum likelihood estimation using the covariance-variance matrix (Grace 2006). P-values for model fit are based on chi-square values. Coefficients presented are standardized values. Sample size was 80 for all models.

Model assumptions and variable transformations.—For all models, the homogeneity of variance was evaluated with Levene’s test and log transformations were applied when necessary to meet the assumption of normally distributed residuals for all general linear models and bivariate normality for structural equation mod-

Table 1. F-statistics and p-values for the effects of treatments (nitrogen, water, and their interaction) and species identity (*Loudetiopsis* or *Tristachya*) on resin ammonium and nitrate, soil-extractable ammonium and nitrate, pH, Al, K, and Ca.

Variable	Nitrogen		Water		Water × Nitrogen		Species	
	F	p	F	p	F	p	F	p
Resin NH ₄ ⁺	<0.01	0.98	4.72	↑0.03*	1.16	0.28	0.18	0.67
Resin NO ₃ ⁻	<0.01	0.99	3.70	↑0.06†	0.83	0.37	0.01	0.93
Soil NH ₄ ⁺	0.75	0.39	0.21	0.65	0.12	0.73	0.53	0.47
Soil NO ₃ ⁻	3.42	↑0.07†	3.76	↓0.06†	3.03	↓0.08†	0.01	0.94
pH	0.38	0.54	0.05	0.83	0.10	0.75	2.24	0.14
Aluminum	1.60	0.21	1.09	0.30	0.89	0.35	0.74	0.39
Potassium	3.00	↓0.09†	0.08	0.77	0.42	0.52	2.49	0.12
Calcium	3.15	↑0.08†	0.09	0.76	0.36	0.55	0.01	0.93

Notes: Arrows to denote the direction of the effect, either increase (↑) or decrease (↓) for $p < 0.10$. Degrees of freedom and sample size: pH, Al, K, and Ca: N = 80 and df = 1, 75. Soil NH₄⁺ and NO₃⁻: N = 99, df = 1, 94. Resin NH₄⁺ and NO₃⁻: N = 76, df = 1, 71.

† $p < 0.10$.

* $p < 0.05$.

els. For all transformations and non-normal distributions (gamma, binomial, and negative binomial) we report back-transformed means and 95% confidence intervals. Structural equation models were analyzed in R 2.10.1 (sem package). All other analyses were performed in SAS 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

Soil responses

Overall, water addition had significant effects on soil phosphorus and mineralized (resins) and inorganic nitrogen pools (bulk soil). On the other hand, the only significant effect of nitrogen addition was an increase in bulk nitrate pools. There was also little indication of increasing soil acidity, aluminum concentrations, or cation leaching with added N, though there was a slight trend towards increased calcium and decreased potassium (Table 1).

Water addition led to increased resin-available ammonium and nitrate over a 28-day period during the peak of the growing season (Table 1). Resin-available ammonium values (controls: $0.84 \pm 0.148 \mu\text{g N}$ [mean \pm SE]) tended to be higher than nitrate values (controls: $0.632 \pm 0.196 \mu\text{g N}$). Resin-available NO₃⁻ increased 69% (H₂O addition: $1.07 \pm 0.176 \mu\text{g N}$) while resin NH₄⁺ increased 71% (H₂O addition: $1.44 \pm 0.175 \mu\text{g N}$) with added water. N addition and species identity did not significantly affect resin-available N of either ionic form (Table 1).

In contrast to results for resin NO₃⁻, water

addition had a marginally significant negative effect on bulk soil NO₃⁻ pools compared to control values (control: 0.105 ± 0.019 , H₂O: 0.103 ± 0.019 ; Fig. 2, Table 1). Soil pools of NO₃⁻ almost doubled with nitrogen addition as compared to controls levels (N addition: $0.206 \pm 0.038 \mu\text{g N/g}$; Fig. 2, Table 1). However, when water was added with nitrogen NO₃⁻ concentrations decreased to levels below control values (N and H₂O addition: $0.104 \pm 0.019 \mu\text{g N/g}$; Fig. 2) and similar to values for plots receiving only the water treatment, suggesting an interactive effect of the two treatments ($p = 0.08$; Table 1). Water and nitrogen addition did not significantly affect bulk soil NH₄⁺ (Table 1). Bulk soil NH₄⁺ values (controls: $2.493 \pm 0.068 \mu\text{g N/g}$) were orders of magnitude greater than NO₃⁻ levels (control: $0.105 \pm 0.019 \mu\text{g N/g}$). Species identity did not have a significant effect on bulk soil NO₃⁻ or NH₄⁺ (Fig. 2, Table 1).

Soil phosphorus was significantly affected by water addition but the direction of the effect varied by focal plant species (Fig. 3). Water addition led to a slight decrease in soil P in soils associated with *Loudetiopsis* (0.104 ± 0.010 , control: $0.115 \pm 0.013 \text{ g/kg}$) while soil P increased in *Tristachya* plots receiving added water (0.151 ± 0.017 , controls $0.117 \pm 0.013 \text{ g/kg}$). P increased in soils associated with both species with N addition (Fig. 3, *Loudetiopsis*: 0.154 ± 0.022 , *Tristachya*: $0.138 \pm 0.019 \text{ g/kg}$). For *Tristachya*, the combination of N and water addition led to greater increases in soil P—42% over control values ($0.168 \pm 0.027 \text{ g/kg}$)—than with N alone.

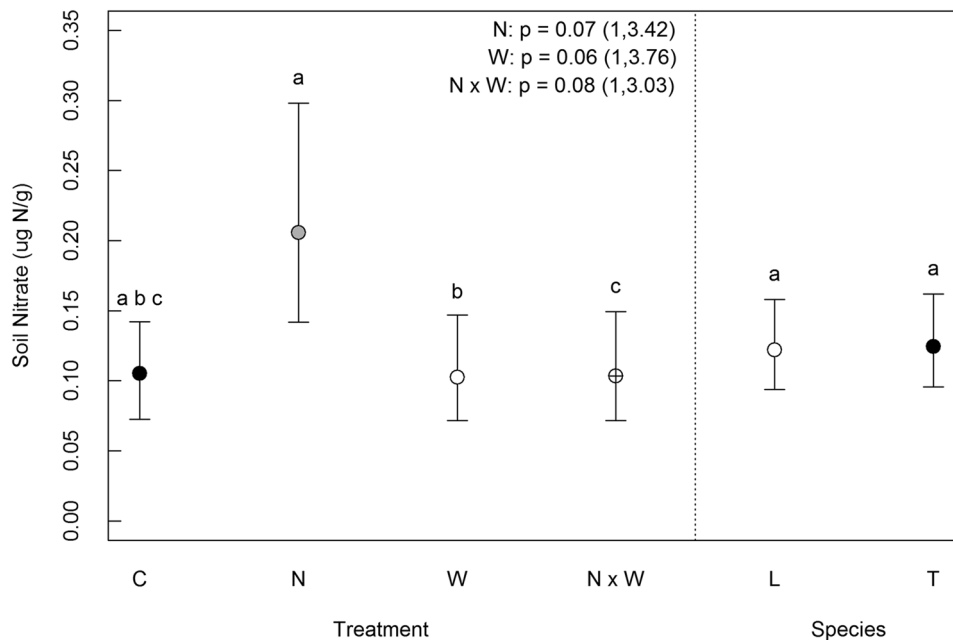


Fig. 2. Effect of treatments and species on soil nitrate ($\mu\text{g}/\text{kg}$) ($N = 98$ and $df = 1, 94$). Treatments significant at the $p < 0.10$ level are indicated by different lower-case letters with the p -value indicated in the figure and numerator degrees of freedom and F -value in parentheses. Bars represent 95% confidence intervals.

However, soil P concentrations in *Loudetiopsis* associated soils with the combination of water and N were lower than control values (0.115 ± 0.016) and similar to soil P values for plots receiving only added water (Fig. 3).

Cations susceptible to leaching (Ca, K) were mildly affected by N addition but were not affected by water addition or species identity. Indicators of soil acidity (pH) and toxicity (Al) were not affected any nitrogen or water addition or species identity (Table 1). N addition led to marginal decreases in K availability (control: 8.36 ± 0.04 , N: 8.23 ± 0.06 g/kg) and marginal increases in Ca (control: 0.0102 ± 0.001 , N: 0.0157 ± 0.004 g/kg) but did not affect pH (control: 4.97 ± 0.05 , N: 4.92 ± 0.04) or Al (control: 0.061 ± 0.002 , N: 0.067 ± 0.003). Water addition and the combined treatment did not significantly affect any of the fertility variables (Table 1).

Plant responses

Water and N-addition effects on foliar nutrients were highly variable among the two species. Foliar P and N:P was affected by treatments but no significant change in foliar N was observed

with any species and treatment combination. *Loudetiopsis* foliar P concentration decreased slightly for all treatments, however only the interaction between water and nitrogen approached significance ($p = 0.09$, Table 2). In contrast, there was a trend towards increased foliar P with *Tristachya* for all treatments—with N or water addition leading to 8% more foliar P over controls and 14% greater foliar P where both N and water were combined (Table 2). Nitrogen:phosphorus ratios (N:P) in *Loudetiopsis* increased slightly with all treatments, though only N addition had a marginally significant effect ($F = 3.74$, $p = 0.06$) with N:P ratios raising to 10.5 ± 2.0 (mean \pm SD) from 9.5 ± 1.4 control values. In contrast, water addition significantly decreased *Tristachya* N:P ratios ($p = 0.02$, $df = 1$, $F = 5.87$) to 9.0 ± 1.3 from 9.9 ± 1.7 control values.

There was a significant interactive effect of the N and water treatments on the likelihood that *Loudetiopsis* would flower ($p = 0.03$, $df = 1$, $F = 4.60$): 95% of the plants receiving both treatments flowered compared to 80% with N, 65% with water, and 90% for controls (Table 3). With *Tristachya*, only water addition had an effect: the percent of flowering individuals increased

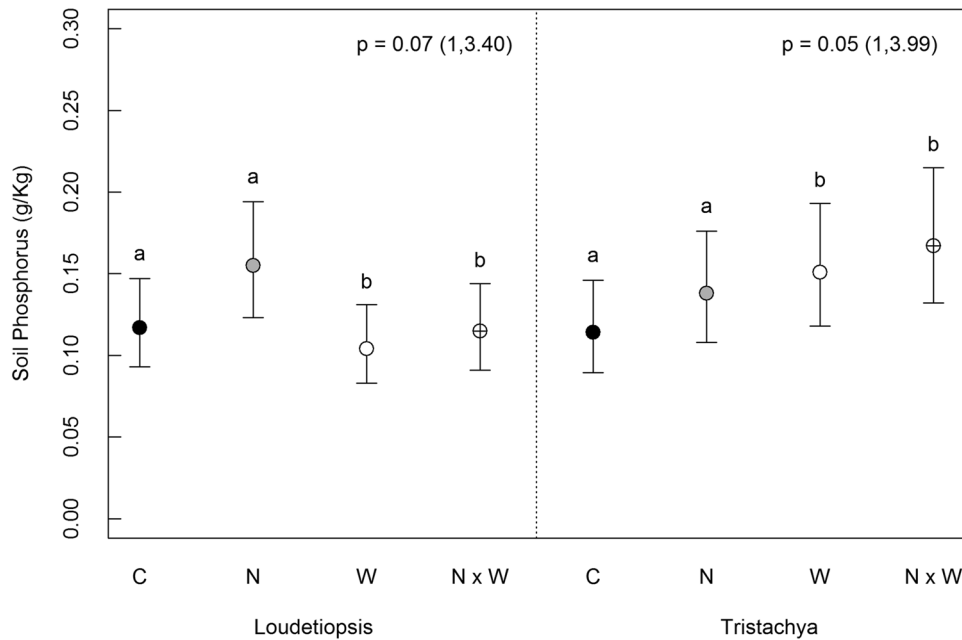


Fig. 3. Soil phosphorus (Mehlich P g/kg) in plots associated with each focal species (*Loudetiopsis*: AIC = -131.42, $\chi^2 = 5.75$ /df = 0.16, *Tristachya*: AIC = -114.17, $\chi^2 = 6.18$ χ^2 /df = 0.17, N = 40 and df = 1, 36 for both species). Treatments significant at the $p < 0.10$ level are indicated by different lower-case letters with the p-value indicated in the figure and numerator degrees of freedom and F-value in parentheses. Bars represent 95% confidence intervals.

from 45% for controls to 70% with either water or water and nitrogen (Table 3). Contrary to expectations, higher resin available ammonium values were correlated with decreasing flowering probability for *Tristachya* ($p = 0.02$, Wald $\chi^2 = 5.44$), while neither form of resin available nitrogen was a significant predictor of flowering likelihood for *Loudetiopsis*. *Loudetiopsis* individuals that did flower produced approximately double the number of flowering tillers with water and the combined N and water treatments

(Table 3). None of the treatments significantly affected the number of flowering tillers produced by *Tristachya* individuals ($p > 0.10$), however the mean number of flowering tillers is also very low for this species (2.0 ± 1.0 flowering tillers/individual in controls).

For the plants that did flower, we also tested whether the nitrogen and water treatments influenced measures of total reproductive effort—the total number of spikelets produced per flowering tiller—and allocation of spikelets per

Table 2. F-statistics and p-values for effects of treatments (nitrogen, water, and their interaction) and covariate live leaf biomass on foliar N and P and N:P ratio.

Variable	Nitrogen				Water				Water × Nitrogen				Live leaf biomass			
	<i>Loudetiopsis</i>		<i>Tristachya</i>		<i>Loudetiopsis</i>		<i>Tristachya</i>		<i>Loudetiopsis</i>		<i>Tristachya</i>		<i>Loudetiopsis</i>		<i>Tristachya</i>	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Foliar N	1.62	0.21	0.40	0.53	0.39	0.53	0.69	0.41	1.35	0.25	0.24	0.63	4.77	↓0.03*	0.07	0.79
Foliar P	1.78	0.19	2.93	↑0.09†	1.17	0.28	3.12	↑0.08†	3.01	↓0.09†	0.02	0.88	1.96	0.17	0.21	0.65
Foliar N:P	3.74	↑0.06†	1.57	0.21	1.70	0.20	5.87	↓0.02*	1.18	0.28	<0.01	0.99	7.34	↓0.01*	0.02	0.90

Notes: Symbols are as in Table 1. Degrees of freedom and sample size: *Loudetiopsis*: N = 80, df = 1, 75; *Tristachya*: N = 79, df = 1, 74.

Table 3. F-statistics and p-values for effects of treatments (nitrogen, water, and their interaction) on reproductive variables by species.

Variable	Nitrogen				Water				Water × Nitrogen			
	<i>Loudetiopsis</i>		<i>Tristachya</i>		<i>Loudetiopsis</i>		<i>Tristachya</i>		<i>Loudetiopsis</i>		<i>Tristachya</i>	
	F	p	F	p	F	p	F	p	F	p	F	p
Flowering probability‡	1.07	0.30	<0.01	0.99	<0.01	0.99	4.99	†0.03*	4.60	†0.03*	<0.01	0.99
Tillers/individual	1.41	0.24	0.77	0.38	5.07	†0.03*	<0.01	0.97	3.85	†0.05†	0.81	0.37
Spikelets/individual	1.71	0.20	0.01	0.92	4.77	†0.03*	0.91	0.35	1.16	0.29	2.14	0.15
Spikelets/tiller	3.32	†0.07†	0.42	0.52	0.11	0.74	1.75	0.19	0.88	0.35	0.19	0.67

Notes: Symbols are as in Table 1. Degrees of freedom and sample size: Flowering probability: *Loudetiopsis*: N = 80, df = 1, 75; *Tristachya*: N = 79, df = 1, 74. Tillers/individual: *Loudetiopsis*: N = 66, df = 1, 62; *Tristachya*: N = 46, df = 1, 42. Spikelets/individual and spikelets/tiller: *Loudetiopsis*: N = 66, df = 1, 62; *Tristachya*: N = 43, df = 1, 39.

‡ Test statistic is Wald χ^2 for the binomial distribution.

flowering tiller. In *Loudetiopsis*, water addition had a significant positive effect on the total number of spikelets: watered plants produced 120% more spikelets on average than control plants (Table 3). Nitrogen addition did not affect total reproductive effort in *Loudetiopsis*, but it did lead to a marginal increase in the allocation of spikelets to each tiller (Table 3). There was no significant difference in either total spikelets per plant or spikelets per flowering tiller with treatments for *Tristachya* ($p > 0.10$).

As with reproductive measures, growth, allocation, and senescence responses to treatments varied between the two focal species. While *Loudetiopsis* diameter growth was not affected by either water or N addition, *Tristachya* diameter did increase significantly with nitrogen addition (Table 4). For both species, growth decreased significantly with plant diameter and increased with higher photosynthetic active radiation (Table 4). *Loudetiopsis* root : shoot ratio decreased with treatments, but there were no significant treatment effects on *Tristachya* root : shoot ratio. Nitrogen and water addition both led to decreases in *Loudetiopsis* root : shoot ratios, leading to an additive decrease of about 45% in ratios in the N × water treatment compared to controls (Table 4). Water addition did lead to approximately 28% higher density of green leaves in the dry season for *Loudetiopsis* but neither treatment significantly affected *Tristachya* leaf senescence. The increase in dry-season green leaf density was also significantly correlated with increased dead aboveground biomass at the end of the experiment for *Loudetiopsis* ($p = 0.04$, $F = 4.34$).

Structural equation models

Structural models were used to test for support of two a priori competing hypotheses (positive vs. negative) for the effects of N deposition and dry-season precipitation change on plant growth and reproduction via changes in soil fertility and plant nutrient status or leaf senescence in the dry season (both hypotheses represented in paths in Fig. 1). The positive plant-soil feedback model tested for the potential of N deposition and water addition to increase soil N (resin nitrate and ammonium) and foliar nutrients (N:P) and for water addition to reduce leaf senescence with positive effects on plant growth and reproduction. This hypothesized model of relationships between variables was supported by the data (model could not be rejected, $\chi^2 = 16.9$, $df = 24$, $p = 0.86$, paths significant at $p < 0.05$: water addition → resin nitrate, species → leaf senescence, flowering culms, foliar N:P, resin ammonium → foliar N:P). However, there was no support for the negative plant-soil feedback model incorporating the potential for N addition to have toxic effects on soils via increasing soil acidity and pH and increased toxic Al ($\chi^2 = 66.08$, $df = 18$, $p < 0.01$).

We also incorporated the results of the ANOVA tests of treatment effects into a final a posteriori SEM for the treatment effects on plant and soils. We retained phosphorus as the only soil fertility factor in the final model because ANOVA tests consistently found significant effects of treatments on plant and soil phosphorus variables but weakly (N, Ca, K, pH, Al) or equivocally supported (some soil N results significant others not significant) treatment effects on other soil variables. We included direct

Table 4. F-statistics and p-values for effects of treatments (nitrogen, water, and their interaction) on plant growth (covariates are initial diameter and light – PAR), allocation to roots vs. shoots, and senescence as indicated by dry-season live leaf density (covariate is plant diameter).

Variable	Nitrogen				Water				Water × Nitrogen			
	<i>Loudetiopsis</i>		<i>Tristachya</i>		<i>Loudetiopsis</i>		<i>Tristachya</i>		<i>Loudetiopsis</i>		<i>Tristachya</i>	
	F	p	F	p	F	p	F	p	F	p	F	p
Diameter growth ‡	1.58	0.21	4.94	†0.03*	0.01	0.94	0.15	0.69	0.54	0.47	0.03	0.86
Root:shoot	3.06	‡0.08†	0.18	0.67	4.16	‡0.04*	0.02	0.88	0.01	0.91	<0.01	0.99
Dry-season live leaf density §	0.48	0.49	0.02	0.88	4.80	†0.03*	0.23	0.63	0.32	0.95	0.03	0.87

Notes: Symbols are as in Table 1. Degrees of freedom and sample size: Diameter growth: *Loudetiopsis*: N = 78, df = 1, 72; *Tristachya*: N = 79, df = 1, 73. Root : shoot: *Loudetiopsis*: N = 80, df = 1, 76; *Tristachya*: N = 79, df = 1, 75. Dry-season live leaf density: Both species: N = 80, df = 1, 75.

‡ Covariates: *Loudetiopsis*: Initial diameter: F-value: 6.62, p = ‡0.01; *Tristachya*: Initial diameter: F-value: 51.93, p = ‡ < 0.01. PAR: *Loudetiopsis*: F-value: 8.63, p = ‡0.01; *Tristachya*: F-value: 6.79, p = ‡0.01.

§ Covariates: *Loudetiopsis*: Diameter: F-value: 6.99, p = ‡0.01; *Tristachya*: Diameter: F-value: 24.93, p = ‡ < 0.01.

pathways between our treatments and plant response variables because ANOVA results suggested that effects, such as the effect of N addition on growth, were uncorrelated with measured treatment effects on soil variables. The final model including P as the only soil variable was strongly supported by the data ($\chi^2 = 7.87$, df = 14, p = 0.89; Fig. 4). Species exerted strong, largely significant, effects (indicated by relatively high standardized coefficient values) on all response variables but diameter change in the final model (Fig. 4). In contrast, the water and nitrogen treatments had strong, but not significant, effects on different response variables (e.g., N on soil P, W on number of flowering culms and senescence, Fig. 4). Light was justified as an important covariate in plant response to the treatments for both species with a strong significant positive effect on plant diameter growth (Fig. 4).

DISCUSSION

Our results support the hypothesis that nitrogen enrichment and dry season water addition at rates projected under global change scenarios could alter the growth and reproduction of Cerrado C-4 grasses. However, both experimental treatments influenced primarily soil and foliar phosphorous, and not the levels of soil and foliar nitrogen as we predicted. In addition, species identity strongly influenced the direction and nature of plant and soil responses despite the relative similarity of our co-dominant study species (C-4 grasses in the same tribe).

Contrary to our expectations, our N addition treatment did not significantly increase resin-available N and had only a weak positive effect on bulk soil nitrate. However, we did observe slight increases in resin-available N of both ion forms with the water addition treatment, a response we expected based on increases in nitrogen mineralization and nitrification with increased dry season precipitation in tropical savannas (Augustine and McNaughton 2004, Bustamante et al. 2006) and tropical dry forests (Davidson et al. 1993). The conflicting effects of water addition on inorganic N—a decrease in soil nitrate coupled with increases in resin ammonium and nitrate—could be due to increased plant uptake of mineralized N in the dry season (Bustamante et al. 2006). Our inability to detect consistent positive effects of our N addition on resin-available N—despite the low levels of available N in the soil (mean: 2.58, lower 95% CL: 1.79, upper 95% CL: 3.77 $\mu\text{g NO}_3^- + \text{NH}_4^+/\text{g}$)—could be explained by soil or litter immobilization (Aber et al. 2002), gaseous losses (Pinto et al. 2006), or leaching to deeper soil layers (Lilienfein et al. 2003). Because our soil N measurements took place on in the wet season, we cannot exclude the possibility that our N addition might significantly increased soil N during other seasons.

The N addition treatment in this study was not linked to strong growth or reproductive responses of the focal C-4 bunchgrasses. Only a weak growth response for *Tristachya* was observed. However, as a functional group, C-4 grasses are known to predict the effects of N-deposition on

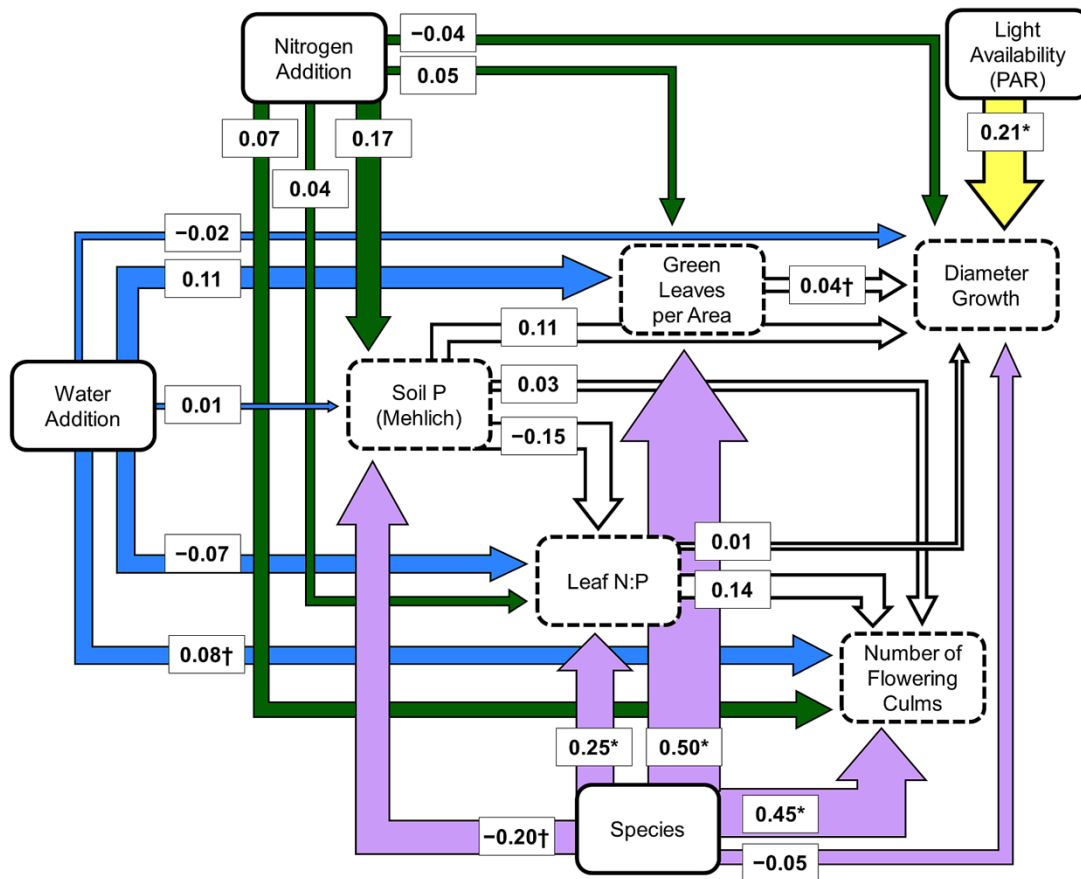


Fig. 4. Final structural equation model for relationships between water addition (blue arrows), nitrogen addition (dark green arrows), species (purple arrows), photosynthetically available radiation (yellow arrow), soil phosphorus (hollow arrows), and plant response variables (hollow arrows). Plant and soil response (endogenous) variables are denoted by dashed outlines and predictor (exogenous) variables with solid outlines. Arrows presented correspond to covariance variables in the model with thickness corresponding to standardized coefficient values. Standardized coefficients are also reported in boxes with significance indicated by * $p < 0.05$, † $p < 0.10$.

other grassland ecosystems (Clark et al. 2007) and bunchgrasses are known to respond to N addition, particularly through tiller recruitment (Tomlinson and O'Connor 2004). Relatively high N use efficiency among C-4 grasses (Craine et al. 2002, Reich et al. 2003, Hikosaka 2004) could explain the weak response to N addition if the trait led to a reduced uptake rates of added N. Both species have low N:P ratios (control *Loude-tiopsis*: 9.43 ± 1.16 [mean \pm SE], *Tristachya*: 9.74 ± 1.17), which can be indicative of nitrogen limitation (Tessier and Raynal 2003). However, the lack of fertilization response we observed is consistent with recent analyses that suggest that

N:P ratios do not necessarily predict absolute nutrient limitation in tropical ecosystems (secondary tropical forest: Davidson et al. 2004; tropical forests: Townsend et al. 2007; African savannas: Craine et al. 2008).

Finally, the lack of response observed could be due to the low amount of N added to simulate N-deposition in this study ($25 \text{ kg ha}^{-1} \text{ yr}^{-1}$). However, our N-addition was in addition to ambient N-deposition which likely exceeds a previous estimate of $9.5 \text{ ha}^{-1} \text{ yr}^{-1}$ reported close to the study area (Lilienfein and Wilcke 2004). Though our N treatment is low compared to the amounts added in many short-term global

change studies (Henry et al. 2006: 70 kg ha⁻¹ yr⁻¹, Bradford et al. 2007: 50 kg ha⁻¹ yr⁻¹, Vourlitis et al. 2007: 50 kg ha⁻¹ yr⁻¹) it is well within the range of values for observed N-deposition impacts (Fenn et al. 2003, Stevens et al. 2004, Baez et al. 2007).

Soil acidity and cation concentrations were not as sensitive to our treatments as resin N and soil P, and the changes that we did observe were not correlated with negative treatment effects on plant growth and reproduction, suggesting that negative soil-feedbacks are not responsible for the weak responses of our focal species to N addition. These results were highlighted by the poor fit of the structural equation model which related Al, P, and soil pH to plant response to treatments. While N addition decreased soil pH and increased Al concentration—indicators of soil acidity—these differences were statistically insignificant and uncorrelated with a detectable decrease in soil P. The slight increase in calcium and decrease in potassium with the N treatment did not support our original expectation that both nutrients would decrease with N as a result of increasing acidity. Overall, our results do not support the assertion that nutrient-poor tropical ecosystems might experience increased soil toxicity as a result of short-term N addition (Matson et al. 1999), or that low cation exchange capacity is related to sensitivity to N addition (Clark et al. 2007). However, these short-term experimental results do not preclude the possibility that long-term N deposition could lead to N leaching and soil acidity—particularly if the N levels surpass plant and microbe capacity to immobilize N (Aber et al. 1998) or if the negative effects on plants and soils have non-linear, and increasingly negative, effects over time (Clark and Tilman 2008).

In contrast to the weak relationship between plant responses and soil nitrogen, soil and plant phosphorus were altered by our treatments. The significant, though divergent, changes in foliar P for both species—increases for *Tristachya*, decreases for *Loudetiopsis*—were linked to species-specific effects on soil P. These divergent species responses could suggest species differences in phosphorus demand and uptake rates with different global change factors. In a grassland global change experiment in California, USA, changes in phosphatase and foliar P concentra-

tion suggested that plant demand for phosphorus decreased with water addition and increased with nitrogen addition (Menge and Field 2007). In this study, the decrease in *Loudetiopsis* foliar P and associated soils with N addition and water addition suggested increased P demand and limitation. In contrast, the increased phosphorus in *Tristachya*-associated soils and increased foliar P with both water and nitrogen addition suggest that P limitation in this species was reduced by both treatments. Additional experiments are needed to elucidate the species-specific mechanisms, such as increased phosphatase activity (Menge and Field 2007), changes in plant P uptake, plant carbon investment in mycorrhizae (Treseder 2004), or more complex plant-microbe feedbacks (Bever et al. 2010) that might explain these results.

While the results of the structural equation models generally support positive soil-plant feedbacks in our study system, the effects of nitrogen and water addition depended on the focal species and the specific growth or reproductive response measured. While growth was enhanced by nitrogen but not water, the inverse was observed for reproduction. Water addition led to high flowering probability and increased numbers of spikelets and potentially seed number for *Loudetiopsis*, while nitrogen addition was negatively correlated with flowering for *Tristachya* and uncorrelated with reproductive traits in *Loudetiopsis*. This result contrasts with previous research that strongly links N addition with higher rates of grass flowering in a temperate grassland (Silletti et al. 2004).

The significant positive interaction of nitrogen and water on flowering probability in this study highlights the importance of testing for the interactive effects of global change factors and indicates that nitrogen deposition could have more positive effects on plant growth and reproduction when combined with increasing dry season rainfall in this ecosystem. Interactive effects of treatments were observed for multiple plant responses including foliar P, tiller number in *Loudetiopsis*, and leaf senescence in *Loudetiopsis*. However, there were also cases where the effects of the two treatments appeared to be simply additive (e.g., decreased root : shoot ratio with *Loudetiopsis*).

Overall, the most important interactive effect

observed was the difference in treatment effects on plant-soil feedbacks by species. We hypothesized that the two C-4 grass species used in the experiment would respond similarly to the experimental manipulations. However, the responses of *Tristachya* and *Loudetiopsis* were idiosyncratic, and species identity explained a larger proportion of the variation in the data than our treatments did in structural equation models (Fig. 2). These species-specific responses, comparable to those observed in other studies of co-occurring C-4 grasses (Silletti and Knapp 2001, Silletti et al. 2004, Fynn et al. 2005), also suggest particular species traits which could be responsible for the differences in response to simulated global change factors. The most notable of these is drought tolerance, which could influence leaf senescence response to water addition (see also Swemmer et al. 2006). Of the two species, *Tristachya*, which had lower rates of leaf senescence, may be more tolerant of drought since its roots are larger in diameter and exploit deeper soil layers than those of *Loudetiopsis*. Such complementary root structure is known to allow co-occurring perennial grasses to exploit different nutrient and water sources in other ecosystems (Fargione and Tilman 2005). Future research would benefit from an explicit understanding of the hydrological niches (Araya et al. 2011) for *Tristachya* and *Loudetiopsis*, a potential mechanism for coexistence of these savanna grasses, and perhaps a predictor of their responses to changing precipitation patterns.

Fire and herbivory—not manipulated in our study—could interact with changing water and nutrient regimes and are likely to influence growth and reproduction of our focal species and other Cerrado plants. For example, the reduction in the proportion of flowering individuals between year one (prior to fertilization) and year two of the study (*Tristachya*: 94–58%, *Loudetiopsis*: 99–83%) could have been related to a fire in the study area in 2006 (Vasconcelos et al. 2009) since Neotropical savanna grasses tend to increase flowering in response to fire (Sarmiento 1992, Baruch and Bilbao 1999). Insect herbivory, which we also did not manipulate, can also have profound effects on ecosystem nutrient cycling in the Cerrado. For example, the effects of leaf-cutter ants on both plant and soil N (Sternberg et al. 2007, Costa et al. 2008, Mundim et al. 2009) are

analogous to the impacts that large migrating ungulates have on N availability in African savannas (Augustine 2003, Holdo et al. 2007, Cech et al. 2008). How both fire and herbivory interact with global change factors could be crucial to predicting soil-plant feedbacks with climate change and rising N deposition rates in the Cerrado in the coming decades.

While experimental precipitation elicited more plant and soil responses than nitrogen addition in this study, we cannot exclude the possibility that ambient N deposition in our study area ($9.5 \text{ kg ha}^{-1} \text{ yr}^{-1}$, 1997–99, Lilienfein and Wilcke 2004) affected our results by reducing ecosystem N limitation. Lack of detailed information on current ambient N deposition rates is a common limitation of tropical N enrichment studies (Bobbink et al. 2010). Future research should endeavor to measure and quantify the effects of background N deposition in Neotropical savannas like the Cerrado which are experiencing rapidly rising N deposition rates.

Conclusions

Our results suggest that the increases in nitrogen deposition and precipitation predicted for the Cerrado region can have interactive positive effects on the growth and reproduction of two dominant grass species via decreased nutrient and water limitation. Contrary to our expectations, the responses varied strongly among species and were largely related to feedbacks between plant and soil phosphorus rather than nitrogen. Our results demonstrate strong species effects on plant-soil feedbacks and suggest that species-specific responses to global changes could exert significant effects on ecosystem properties. Such a result is problematic for predicting global change effects in Neotropical savannas given extremely high plant species richness and diverse strategies for nutrient acquisition (Bustamante et al. 2004, Townsend et al. 2008). Future experiments, conducted over longer time-scales and with different Cerrado functional groups, are necessary to determine if the effects observed in this study are representative of long-term ecosystem responses to changes in precipitation and chronic N deposition. We suggest that structural equation models can offer unique perspectives to analysis of complex global change studies (Clark et al. 2007, e.g., Antoninka

et al. 2009) because they can test a priori models for direct and indirect interactions between treatments, soils, and plants as well as provide a posteriori models for complex interactions that may motivate future research. Finally, our findings emphasize that global change factors should be simultaneously manipulated at realistic levels in future experiments because of their potential to have complex non-additive effects on tropical savanna plants and soils.

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LITERATURE CITED

- Aber, J., W. McDowell, K. Nadelhoffer, A. Magill, G. Berntson, M. Kamakea, S. McNulty, W. Currie, L. Rustad, and I. Fernandez. 1998. Nitrogen saturation in temperate forest ecosystems—Hypotheses revisited. *BioScience* 48:921–934.
- Aber, J. D., S. V. Ollinger, C. T. Driscoll, G. E. Likens, R. T. Holmes, R. J. Freuder, and C. L. Goodale. 2002. Inorganic nitrogen losses from a forested ecosystem in response to physical, chemical, biotic, and climatic perturbations. *Ecosystems* 5:648–658.
- Antoninka, A., J. E. Wolf, M. Bowker, A. T. Classen, and N. C. Johnson. 2009. Linking above- and belowground responses to global change at community and ecosystem scales. *Global Change Biology* 15:914–929.
- Araya, Y. N., J. Silvertown, D. J. Gowing, K. J. McConway, H. P. Linder, and G. Midgley. 2011. A fundamental, eco-hydrological basis for niche segregation in plant communities. *New Phytologist* 189:253–258.
- Augustine, D. J. 2003. Spatial heterogeneity in the herbaceous layer of a semi-arid savanna ecosystem. *Plant Ecology* 167:319–332.
- Augustine, D. J., and S. J. McNaughton. 2004. Temporal asynchrony in soil nutrient dynamics and plant production in a semiarid ecosystem. *Ecosystems* 7:829–840.
- Austin, A. T., L. Yahdjian, J. M. Stark, J. Belnap, A. Porporato, U. Norton, D. A. Ravetta, and S. M. Schaeffer. 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. *Oecologia* 141:221–235.
- Baez, S., J. Fargione, D. I. Moore, S. L. Collins, and J. R. Gosz. 2007. Atmospheric nitrogen deposition in the northern Chihuahuan desert: Temporal trends and potential consequences. *Journal of Arid Environments* 68:640–651.
- Barger, N. N., C. M. D'Antonio, T. Ghneim, K. Brink, and E. Cuevas. 2002. Nutrient limitation to primary productivity in a secondary savanna in Venezuela. *Biotropica* 34:493–501.
- Baruch, Z., and B. Bilbao. 1999. Effects of fire and defoliation on the life history of native and invader C-4 grasses in a Neotropical savanna. *Oecologia* 119:510–520.
- Bever, J. D., I. A. Dickie, E. Facelli, J. M. Facelli, J. Klironomos, M. Moora, M. C. Rillig, W. D. Stock, M. Tibbett, and M. Zobel. 2010. Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology and Evolution* 25:468–478.
- Bobbink, R., et al. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications* 20:30–59.
- Borken, W., and E. Matzner. 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Global Change Biology* 15:808–824.
- Bradford, M. A., H. B. Schumacher, S. Catovsky, T. Eggers, J. E. Newington, and G. M. Tordoff. 2007. Impacts of invasive plant species on riparian plant assemblages: interactions with elevated atmospheric carbon dioxide and nitrogen deposition. *Oecologia* 152:791–803.
- Bustamante, M. M. C., L. A. Martinelli, D. A. Silva, P. B. Camargo, C. A. Klink, T. F. Domingues, and R. V. Santos. 2004. ¹⁵N natural abundance in woody plants and soils of central Brazilian savannas (cerrado). *Ecological Applications* 14:200–213.
- Bustamante, M. M. C., E. Medina, G. P. Asner, G. B. Nardoto, and D. C. Garcia-Montiel. 2006. Nitrogen cycling in tropical and temperate savannas. *Biogeochemistry* 79:209–237.

- Cardoso, E. M. I. Cruzeiro Moreno, E. M. Bruna, and H. L. Vasconcelos. 2009. Vegetation dynamics in the Cerrado: 18 years of ecological succession at Estação Ecológica do Panga (Uberlândia, MG). *Caminhos de Geografia*.
- Carrera, A. L., M. B. Bertiller, C. L. Sain, and M. J. Mazzarino. 2003. Relationship between plant nitrogen conservation strategies and the dynamics of soil nitrogen in the arid Patagonian Monte, Argentina. *Plant and Soil* 255:595–604.
- Cech, P. G., T. Kuster, P. J. Edwards, and H. O. Venterink. 2008. Effects of herbivory, fire and N-2-fixation on nutrient limitation in a humid African savanna. *Ecosystems* 11:991–1004.
- Christensen, J. H., et al. 2007. Regional climate projections. Pages 1–46 in S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK.
- Clark, C. M., E. E. Cleland, S. L. Collins, J. E. Fargione, L. Gough, K. L. Gross, S. C. Pennings, K. N. Suding, and J. B. Grace. 2007. Environmental and plant community determinants of species loss following nitrogen enrichment. *Ecology Letters* 10:596–607.
- Clark, C. M., and D. Tilman. 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature* 451:712–715.
- Cleland, E. E., N. R. Chiariello, S. R. Loarie, H. A. Mooney, and C. B. Field. 2006. Diverse responses of phenology to global changes in a grassland ecosystem. *Proceedings of the National Academy of Sciences USA* 103:13740–13744.
- Costa, A. N., H. L. Vasconcelos, E. H. M. Vieira-Neto, and E. M. Bruna. 2008. Do herbivores exert top-down effects in Neotropical savannas? Estimates of biomass consumption by leaf-cutter ants. *Journal of Vegetation Science* 19:849–854.
- Craine, J. M., C. Morrow, and W. D. Stock. 2008. Nutrient concentration ratios and co-limitation in South African grasslands. *New Phytologist* 179:829–836.
- Craine, J. M., D. Tilman, D. Wedin, P. Reich, M. Tjoelker, and J. Knops. 2002. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. *Functional Ecology* 16:563–574.
- Cruz, P. 1997. Effect of shade on the growth and mineral nutrition of a C-4 perennial grass under field conditions. *Plant and Soil* 188:227–237.
- Davidson, E. A., C. J. R. de Carvalho, I. C. G. Vieira, R. D. Figueiredo, P. Moutinho, F. Y. Ishida, M. T. P. dos Santos, J. B. Guerrero, K. Kalif, and R. T. Saba. 2004. Nitrogen and phosphorus limitation of biomass growth in a tropical secondary forest. *Ecological Applications* 14:150–163.
- Davidson, E. A., P. A. Matson, P. M. Vitousek, R. Riley, K. Dunkin, G. Garciamendez, and J. M. Maass. 1993. Processes regulating soil emissions of NO and N₂O in a seasonally dry tropical forest. *Ecology* 74:130–139.
- EMPRAPA. 1999. Sistema brasileiro de classificação de solos. Centro Nacional de Pesquisa de Solos, Embrapa Solos, Rio de Janeiro.
- Fargione, J., and D. Tilman. 2005. Niche differences in phenology and rooting depth promote coexistence with a dominant C-4 bunchgrass. *Oecologia* 143:598–606.
- Fay, P. A., J. D. Carlisle, A. K. Knapp, J. M. Blair, and S. L. Collins. 2003. Productivity responses to altered rainfall patterns in a C-4-dominated grassland. *Oecologia* 137:245–251.
- Fenn, M. E., et al. 2003. Ecological effects of nitrogen deposition in the western United States. *BioScience* 53:404–420.
- Fisher, F. M., and W. G. Whitford. 1995. Field simulation of wet and dry years in the Chihuahuan Desert: Soil moisture, N-mineralization, and ion-exchange resin bags. *Biology and Fertility of Soils* 20:137–146.
- Furley, P. A. 2007. Tropical savannas and associated forests: vegetation and plant ecology. *Progress in Physical Geography* 31:203–211.
- Fynn, R. W. S., C. D. Morris, and K. P. Kirkman. 2005. Plant strategies and trait trade-offs influence trends in competitive ability along gradients of soil fertility and disturbance. *Journal of Ecology* 93:384–394.
- Gardner, T. A. 2006. Tree-grass coexistence in the Brazilian cerrado: demographic consequences of environmental instability. *Journal of Biogeography* 33:448–463.
- Grace, J. B. 2006. Structural equation modeling and natural systems. Cambridge University Press, Cambridge, UK.
- Grace, J. B., and B. H. Pugsek. 1998. On the use of path analysis and related procedures for the investigation of ecological problems. *American Naturalist* 152:151–159.
- Gruber, N. G., and J. N. Galloway. 2008. An Earth-system perspective of the global nitrogen cycle. *Nature* 45:293–296.
- Haylock, M. R., et al. 2006. Trends in total and extreme South American rainfall in 1960–2000 and links with sea surface temperature. *Journal of Climate* 19:1490–1512.
- Henry, H. A. L., N. R. Chiariello, P. M. Vitousek, H. A. Mooney, and C. B. Field. 2006. Interactive effects of fire, elevated carbon dioxide, nitrogen deposition, and precipitation on a California annual grassland. *Ecosystems* 9:1066–1075.
- Hikosaka, K. 2004. Interspecific difference in the photosynthesis-nitrogen relationship: patterns,

- physiological causes, and ecological importance. *Journal of Plant Research* 117:481–494.
- Holdo, R. M., R. D. Holt, M. B. Coughenour, and M. E. Ritchie. 2007. Plant productivity and soil nitrogen as a function of grazing, migration and fire in an African savanna. *Journal of Ecology* 95:115–128.
- Instituto de Geografia. 2008. *Alturas Pluviométricas Mensais Uberlândia, MG 2004-2008*. Universidade Federal de Uberlândia, Uberlândia, MG.
- Knapp, A. K., C. E. Burns, R. W. S. Fynn, K. P. Kirkman, C. D. Morris, and M. D. Smith. 2006. Convergence and contingency in production-precipitation relationships in North American and South African C-4 grasslands. *Oecologia* 149:456–464.
- Knapp, A. K., P. A. Fay, J. M. Blair, S. L. Collins, M. D. Smith, J. D. Carlisle, C. W. Harper, B. T. Danner, M. S. Lett, and J. K. McCarron. 2002. Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science* 298:2202–2205.
- Kochy, M., and S. D. Wilson. 2004. Semiarid grassland responses to short-term variation in water availability. *Plant Ecology* 174:197–203.
- Kozovits, A. R., M. M. C. Bustamante, C. R. Garofalo, S. Bucci, A. C. Franco, G. Goldstein, and F. C. Meinzer. 2007. Nutrient resorption and patterns of litter production and decomposition in a Neotropical savanna. *Functional Ecology* 21:1034–1043.
- Lau, J. A., J. Peiffer, P. B. Reich, and P. Tiffin. 2008. Transgenerational effects of global environmental change: long-term CO₂ and nitrogen treatments influence offspring growth response to elevated CO₂. *Oecologia* 158:141–150.
- Lilienfein, J., and W. Wilcke. 2004. Water and element input into native, agri- and silvicultural ecosystems of the Brazilian savanna. *Biogeochemistry* 67:183–212.
- Lilienfein, J., W. Wilcke, L. Vilela, M. A. Ayarza, S. D. Lima, and W. Zech. 2003. Soil fertility under native Cerrado and pasture in the Brazilian savanna. *Soil Science Society of America Journal* 67:1195–1205.
- Ludwig, F., H. de Kroon, H. H. T. Prins, and F. Berendse. 2001. Effects of nutrients and shade on tree-grass interactions in an East African savanna. *Journal of Vegetation Science* 12:579–588.
- Magrin, G., C. Gay García, D. Cruz Choque, J. C. Giménez, A. R. Moreno, G. J. Nagy, C. Nobre, and A. Villamizar. 2007. 2007: Latin America. Pages 581–615 in M. L. Parry, O. F. Canziani, J. P. Palutikof, P. J. van der Linden, and C. E. Hanson, editors. *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK.
- Marty, J. T., S. K. Collinge, and K. J. Rice. 2005. Responses of a remnant California native bunchgrass population to grazing, burning and climatic variation. *Plant Ecology* 181:101–112.
- Matson, P. A., W. H. McDowell, A. R. Townsend, and P. M. Vitousek. 1999. The globalization of N deposition: ecosystem consequences in tropical environments. *Biogeochemistry* 46:67–83.
- Menge, D. N. L., and C. B. Field. 2007. Simulated global changes alter phosphorus demand in annual grassland. *Global Change Biology* 13:2582–2591.
- Missouri Botanical Garden. 2009. w3-TROPICOS. Missouri Botanical Garden, St. Louis, Missouri, USA.
- Mitchell, R. J. 1992. Testing evolutionary and ecological hypotheses using path analysis and structural equation modeling. *Functional Ecology* 6:123–129.
- Mundim, F. M., A. N. Costa, and H. L. Vasconcelos. 2009. Leaf nutrient content and host plant selection by leaf-cutter ants, *Atta laevigata*, in a Neotropical savanna. *Entomologia Experimentalis Et Applicata* 130:47–54.
- Nafus, A. M., M. P. McClaran, S. R. Archer, and H. L. Throop. 2009. Multispecies allometric models predict grass biomass in semidesert rangeland. *Rangeland Ecology and Management* 62:68–72.
- Nardoto, G. B., M. M. D. Bustamante, A. S. Pinto, and C. A. Klink. 2006. Nutrient use efficiency at ecosystem and species level in savanna areas of Central Brazil and impacts of fire. *Journal of Tropical Ecology* 22:191–201.
- Nippert, J. B., P. A. Fay, J. D. Carlisle, A. K. Knapp, and M. D. Smith. 2009. Ecophysiological responses of two dominant grasses to altered temperature and precipitation regimes. *Acta Oecologica, International Journal of Ecology* 35:400–408.
- Oliveira, P. S., and R. J. Marquis, editors. 2002. *The cerrados of Brazil: ecology and natural history of a Neotropical savanna*. Columbia University Press, New York, New York, USA.
- Pandey, C. B., and J. S. Singh. 1992. Rainfall and grazing effects on net primary productivity in a tropical savanna, India. *Ecology* 73:2007–2021.
- Phoenix, G. K., et al. 2006. Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. *Global Change Biology* 12:470–476.
- Pinto, A. D., M. M. C. Bustamante, M. Da Silva, K. W. Kisselle, M. Brossard, R. Kruger, R. G. Zepp, and R. A. Burke. 2006. Effects of different treatments of pasture restoration on soil trace gas emissions in the cerrados of central Brazil. *Earth Interactions* 10.
- Ratter, J. A., J. F. Ribeiro, and S. Bridgewater. 1997. The Brazilian cerrado vegetation and threats to its biodiversity. *Annals of Botany* 80:223–230.
- Reich, P. B., C. Buschena, M. G. Tjoelker, K. Wrage, J. Knops, D. Tilman, and J. L. Machado. 2003.

- Variation in growth rate and ecophysiology among 34 grassland and savanna species under contrasting N supply: a test of functional group differences. *New Phytologist* 157:617–631.
- Sarmiento, G. 1992. Adaptive strategies of perennial grasses in South-American savannas. *Journal of Vegetation Science* 3:325–336.
- Sarmiento, G., M. P. da Silva, M. E. Naranjo, and M. Pinillos. 2006. Nitrogen and phosphorus as limiting factors for growth and primary production in a flooded savanna in the Venezuelan Llanos. *Journal of Tropical Ecology* 22:203–212.
- Schlesinger, W. H. 2009. On the fate of anthropogenic nitrogen. *Proceedings of the National Academy of Sciences USA* 106:203–208.
- Seagle, S. W., and S. J. McNaughton. 1993. Simulated effects of precipitation and nitrogen on Serengeti grassland productivity. *Biogeochemistry* 22:157–178.
- Siemann, E., W. E. Rogers, and J. B. Grace. 2007. Effects of nutrient loading and extreme rainfall events on coastal tallgrass prairies: invasion intensity, vegetation responses, and carbon and nitrogen distribution. *Global Change Biology* 13:2184–2192.
- Silletti, A. M., and A. K. Knapp. 2001. Responses of the codominant grassland species *Andropogon gerardii* and *Sorghastrum nutans* to long-term manipulations of nitrogen and water. *American Midland Naturalist* 145:159–167.
- Silletti, A. M., A. K. Knapp, and J. M. Blair. 2004. Competition and coexistence in grassland codominants: responses to neighbour removal and resource availability. *Canadian Journal of Botany* 82:450–460.
- Soil Survey Staff. 2003. Keys to soil taxonomy. Ninth edition. USDA Natural Resources Conservation Service.
- Sternberg, L. D., M. C. Pinzon, M. Z. Moreira, P. Moutinho, E. I. Rojas, and E. A. Herre. 2007. Plants use macronutrients accumulated in leaf-cutting ant nests. *Proceedings of the Royal Society B* 274:315–321.
- Stevens, C. J., N. B. Dise, J. O. Mountford, and D. J. Gowing. 2004. Impact of nitrogen deposition on the species richness of grasslands. *Science* 303:1876–1879.
- Swemmer, A. M., A. K. Knapp, and M. D. Smith. 2006. Growth responses of two dominant C4 grass species to altered water availability. *International Journal of Plant Sciences* 167:1001–1010.
- Tessier, J. T., and D. J. Raynal. 2003. Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. *Journal of Applied Ecology* 40:523–534.
- Tomlinson, K. W., and T. G. O'Connor. 2004. Control of tiller recruitment in bunchgrasses: uniting physiology and ecology. *Functional Ecology* 18:489–496.
- Townsend, A. R., G. P. Asner, and C. C. Cleveland. 2008. The biogeochemical heterogeneity of tropical forests. *Trends in Ecology and Evolution* 23:424–431.
- Townsend, A. R., C. C. Cleveland, G. P. Asner, and M. M. C. Bustamante. 2007. Controls over foliar N : P ratios in tropical rain forests. *Ecology* 88:107–118.
- Treseder, K. K. 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO₂ in field studies. *New Phytologist* 164:347–355.
- Vasconcelos, H. L., R. Pacheco, R. C. Silva, P. B. Vasconcelos, C. T. Lopes, A. N. Costa, and E. M. Bruna. 2009. Dynamics of the leaf-litter arthropod fauna following fire in a Neotropical woodland savanna. *Plos One* 4.
- Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. *Ecological Applications* 7:737–750.
- Vourlitis, G. L., S. Pasquini, and G. Zorba. 2007. Plant and soil N response of southern californian semi-arid shrublands after 1 year of experimental N deposition. *Ecosystems* 10:263–279.
- Yahdjian, L., O. Sala, and A. Austin. 2006. Differential controls of water input on litter decomposition and nitrogen dynamics in the Patagonian steppe. *Ecosystems* 9:128–141.
- Zavaleta, E. S., M. R. Shaw, N. R. Chiariello, B. D. Thomas, E. E. Cleland, C. B. Field, and H. A. Mooney. 2003. Grassland responses to three years of elevated temperature, CO₂, precipitation, and N deposition. *Ecological Monographs* 73:585–604.
- Zhang, X. B., F. W. Zwiers, G. C. Hegerl, F. H. Lambert, N. P. Gillett, S. Solomon, P. A. Stott, and T. Nozawa. 2007. Detection of human influence on twentieth-century precipitation trends. *Nature* 448:461–464.

SUPPLEMENTAL MATERIAL

APPENDIX

We measured volumetric water content (m^3/m^3) from July to August of 2008 with Soil Moisture Smart Sensors (Onset Computer Corp., Bourne, MA, USA). Sensors were located adjacent to plants in irrigation and control treatments and recorded a measurement every 5 minutes; they were moved to new plants approximately every 10 days. During the study period the average daily volumetric water content (m^3/m^3) in watered plots (mean 0.03, lower 95% CL 0.02, upper 95% CL 0.04) was approximately three times greater than in un-watered plots (mean 0.01, lower 95% CL 0.01, upper 95% CL 0.02). Average, maximum, and minimum daily soil moisture content in watered plots was significantly lower in un-watered plots than watered plots ($p < 0.0001$ for minimum, maximum, and average, F-value: avg.: 66.10, max.: 105.17, min.: 32.82, general linear model with day as a random effect).