PLANT GROWTH AND SOIL RESPONSES TO SIMULATED NITROGEN DEPOSITION AND DRY SEASON PRECIPITATION IN A NEOTROPICAL SAVANNA

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2009

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To the Cerrado
To the hope of a future with wild places and intact ecosystems
To my grandparents, who collectively encouraged intellectual curiosity, compassion for my fellow human beings, and a passion for biodiversity

ACKNOWLEDGMENTS

I am eternally grateful to my committee, Dr. Heraldo Vasconcelos, Dr. Michelle Mack, and my adviser Dr. Emilio Bruna, for their support and advice. A special note of thanks to Dr. John (Jack) Ewel, who graciously agreed to participate in my defense in replacement for an off-campus member. I am indebted to the students of the Laboratório de Insetos Socias, Universidade Federal de Uberlândia, Brazil, for their help and kindness as I navigated Brazilian culture, Portuguese language, and Cerrado ecology in pursuit of my Master's data. I thank the staff of the Laboratório de Análises de Solos e Calcários, Universidade Federal de Uberlândia, who cheerfully guided me through a litany of soil and foliar analyses. I am grateful to numerous professors, staff, and fellow graduate students of the Department of Wildlife Conservation and Ecology and Department of Botany at University of the Florida who were invaluable throughout the process of thesis development, implementation, and completion. Meghan Brennan and James Colee, of the UF Institute of Food and Agricultural Sciences Statistics Consulting Unit provided crucial research design and statistical analysis support. Special thanks to the Bruna Lab and the Mack Lab for feedback, moral support, and encouragement, particularly through the dark period known as "learning how to use the autoanalyzer". I would never have arrived, or succeeded, at the University of Florida without the exemplary training I received Colorado College where I completed my BA. Last, but never least, to my friends and family who have been steadfast in their support for the last two years, and always. This research was supported by the National Science Foundation (Grant DEB 0542287 and the Graduate Research Fellowship Program).

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August 2009

Chair: Emilio M. Bruna
Major: Wildlife Ecology and Conservation

Anthropogenic increases in nitrogen deposition and precipitation change could alter plant growth and biomass allocation, foliar nutrient concentration, and the influence of plant species on soil nutrient composition. The potential responses of Neotropical savanna plant species and soils to global change factors are unknown, despite the possibility of climate feedbacks and reduced biodiversity. We tested how simulated precipitation change and nitrogen deposition would affect the growth and reproduction of the native grasses *Loudetiopsis chrysothrix* and *Tristachya leiostachya* and characteristics of their associated soils in the Brazilian Cerrado. The two species responded differently to water, nitrogen, and their interaction. *Tristachya* was more likely to flower with water addition, whereas *Loudetiopsis* individuals were more likely to flower with both water and nitrogen. *Tristachya* decreased genet diameter growth whereas *Loudetiopsis* increased genet diameter growth with added nitrogen. *Loudetiopsis* dry season leaf senescence decreased with water addition however, none of the treatments affected *Tristachya* leaf senescence. *Loudetiopsis* individuals' root:shoot ratios decreased with added water, but *Tristachya* individuals' root:shoot ratios did not change in any treatment. The foliar phosphorous

of *Tristachya* individuals increased, while *Loudetiopsis* foliar phosphorus decreased with the water and nitrogen treatment. None of the treatments significantly affected foliar nitrogen for either species. Plant-available phosphorus concentration increased in *Loudetiopsis* associated soils with nitrogen addition and increased in *Tristachya* associated soils with the nitrogen and water treatment. These results suggest that interspecific differences in phosphorus acquisition and use could influence Neotropical plant and soil responses to global change factors. Nitrogen addition alone did not affect most reproductive variables, increased the growth of only one species, and did not increase foliar nitrogen. In contrast, water addition affected a wide variety of traits, especially in combination with nitrogen. These results imply that the impacts of nitrogen deposition and precipitation change in the Cerrado may significantly interact, and vary by species.

CHAPTER 1
INTRODUCTION

Anthropogenic nitrogen addition has more than doubled pre-industrial nitrogen inputs to terrestrial environments (reviewed in Schlesinger, 2009). In addition to a suite of soil biogeochemical changes such as increased soil acidity and cation availability (Aber *et al.*, 1998, Vitousek *et al.*, 1997), nitrogen deposition also affects plants by causing them to alter their patterns of growth, biomass allocation, phenology, fitness, and their interactions with other members of the local plant community (Clark & Tilman, 2008, Cleland *et al.*, 2006, Lau *et al.*, 2008). However, the direction and magnitude of these plant responses can also be influenced by other factors including climate and species identity (Craine *et al.*, 2002, Zavaleta *et al.*, 2003). The responses of plants and soil to nitrogen deposition can ultimately change aboveground net primary production (ANPP), decrease plant diversity, and impact climate via negative or positive feedbacks to the carbon cycle (Gruber, 2008, Vitousek *et al.*, 1997).

Human-forced climate change is altering the pattern and abundance of precipitation across the world's surface (Zhang *et al.*, 2007). Plants are particularly sensitive to changes in precipitation patterns because the amount of annual rainfall and its variability exert strong effects on plant growth and phenology (Fay *et al.*, 2003, Kochy & Wilson, 2004, Zavaleta *et al.*, 2003). Precipitation change can affect the nitrogen cycle in xeric systems because nitrogen mineralization and plant demand for nitrogen are often tightly synchronized with seasonal precipitation (Austin *et al.*, 2004, Knapp *et al.*, 2006, Yahdjian *et al.*, 2006). The few experimental studies to date combining nitrogen enrichment and precipitation change (Cleland *et al.*, 2006, Siemann *et al.*, 2007) have documented idiosyncratic responses – additive, dampened, and multiplicative interactive effects on plant flowering and growth (Cleland *et al.*, 2006, Henry *et al.*, 2006, Zavaleta *et al.*, 2003).

Most experimental research evaluating the ecological consequences of global change phenomena has been conducted in the temperate zone (Carrera *et al.*, 2003, Cleland *et al.*, 2006, Fisher & Whitford, 1995, Kochy & Wilson, 2004, Zavaleta *et al.*, 2003). Consequently, the responses of tropical ecosystems – which account for a large proportion of the globe's net primary production and are reservoirs of biodiversity – to global change factors are relatively unknown (Matson *et al.*, 1999). The lack of experimental evidence exists despite the increase of nitrogen deposition in several tropical hotspots (Phoenix *et al.*, 2006) and the recognition that even moist tropical forests could be highly sensitive to precipitation change (Phillips *et al.*, 2006). The research and theory that does address the response of tropical ecosystems to global change focuses primarily on tropical wet forests (Davidson *et al.*, 2007, Matson *et al.*, 1999, Phillips *et al.*, 2009, Vitousek, 1984). However, the tropics include a range of biomes – including savannas, grasslands, and dry forests – and the responses of these ecosystems to nitrogen deposition and altered precipitation regimes are likely to be markedly different.

Vegetation in tropical savannas and grasslands may be particularly sensitive to either increases or decreases in rainfall patterns because of ecosystem dependence on seasonal precipitation (Austin *et al.*, 2004, O'Connor, 1994, Pandey & Singh, 1992, Seagle & McNaughton, 1993). Because nitrogen can limit plant growth in tropical grasslands and savannas (Augustine, 2003, Barger *et al.*, 2002, Sarmiento *et al.*, 2006), nitrogen deposition could increase aboveground productivity. Precipitation changes and nitrogen deposition could interact to influence plant growth because seasonal rainfall patterns can determine the magnitude and timing of nitrogen mineralization (Austin *et al.*, 2004, Seagle & McNaughton, 1993, Yahdjian *et al.*, 2006). The relative importance of precipitation and nitrogen as controls over plant growth can vary with extraneous factors such as successional state, soil fertility, and fire frequency

(Bustamante *et al.*, 2006, Davidson *et al.*, 2004, Harrington *et al.*, 2001, Sarmiento *et al.*, 2006, Vitousek, 1984). The potential ecosystem consequences of the combination of altered precipitation and nitrogen deposition in tropical savannas are unclear, since to my knowledge no research has addressed their interactive effects on savanna plants and soils.

The global significance of tropical savanna response to global change factors is well represented by their conservation value and substantial extent in the Neotropics. Savannas are the second largest Neotropical biome after lowland Neotropical forests, comprising 45 % of the area of South America (Huntley & Walker, 1982). The largest and most biodiverse Neotropical savanna region is the Brazilian Cerrado, which occupies 2 million km^2 and harbors an estimated 10,000 plant species (Oliveira & Marquis, 2002, Ratter *et al.*, 1997). Cerrado species and ecosystem function are increasingly threatened by mechanized agriculture, introduction of non-native grasses, and urbanization (Ratter *et al.*, 1997). The increasing use of nitrogen fertilizer and rising rates of fossil fuel combustion in Brazil's extensive urban areas are increasing nitrogen deposition over wide swathes of the Cerrado from approximately an average of 5 – 13 kg N ha^{-1} yr^{-1} in the 1990's to a projected 14 – 38 kg N ha^{-1} yr^{-1} by the year 2050 (Bustamante *et al.*, 2006, Phoenix *et al.*, 2006).

The effects of nitrogen enrichment on Cerrado plant species is uncertain, as the relative importance of nitrogen and phosphorus limitation can vary by time since fire, species characteristics, and insect herbivore activity (Bucci *et al.*, 2006, Bustamante *et al.*, 2004, Nardoto *et al.*, 2006, Sternberg *et al.*, 2007). For a number of reasons, the Cerrado nitrogen cycle is quite distinct from other tropical savannas. For example, the Cerrado lacks the migrating large ungulates that seasonally increase nitrogen availability (Augustine *et al.*, 2003) and counteract nitrogen loss from frequent grassland fires (Cech *et al.*, 2008, Holdo *et al.*, 2007) in African

savannas. The Cerrado equivalent may be insect herbivory: leaf-cutting ants have been tied to increased soil nitrogen availability (Sternberg *et al.*, 2007) and foliar nutrient content (Mundim *et al.*, 2009). While herbivores like leaf-cutting ants may harvest a significant amount of biomass (Costa *et al.*, 2008), their enriching effects on soil nutrients may be offset by fire (Sousa-Souto *et al.*, 2008). A substantial proportion of aboveground nitrogen and sulfur can be lost in Cerrado fires (Boone Kauffman *et al.*, 1994), though soil nitrogen and other nutrient values may actually be higher in areas with higher fire frequency (da Silva & Batalha, 2008). Native nitrogen fixing plant species and free living microbes may add 16-44 kg N ha^{-1} yr^{-1} to the Cerrado biome as a whole , however their effectiveness may be limited by other macronutrients (Bustamante *et al.*, 2006). Nitrogen availability is also linked to plant water uptake in the Cerrado, as there is some evidence that nitrogen fertilization can affect plant water uptake (Bucci *et al.*, 2006, Scholz *et al.*, 2007), but the possible interactive effects of nitrogen and water on native plant species have not been addressed experimentally.

Because of the uncertainty regarding plant nutrient limitation in the Cerrado, I propose two opposing hypotheses for the effects of dry season water addition and nitrogen addition on plant growth and allocation. Previous research has suggested that Cerrado species may be nitrogen limited (Bustamante *et al.*, 2006). I therefore predicted that nitrogen addition could have a fertilizing effect on plant growth (referred to hereafter as the "Fertilization Hypothesis"). Specifically, I expected that growth and reproduction would increase in response to increasing plant available nitrogen resulting from nitrogen enrichment. Whereas, the effects of precipitation change effects would depend on the direction of rainfall change (increase or decrease) and the timing of rain events (Austin *et al.*, 2004). A majority of IPCC models predict increasing precipitation in the dry season for the Cerrado; I predicted that this could lead to reduced leaf

senescence due to water limitation, higher rates of photosynthesis, and carbon storage. Water addition could also increase growth and reproduction indirectly by mimicking the stimulating effect of precipitation on nitrogen mineralization (Seagle & McNaughton, 1993) .

Although nitrogen and water could limit Cerrado plant growth and reproduction, nitrogen deposition could actually exacerbate soil conditions that inhibit growth (e.g., low cation and phosphorus availability, high acidity, and high aluminum levels (Matson et al., 1999). The result could be growth limitation by macronutrients other than nitrogen (e.g., phosphorus) or aluminum toxicity. Such as response could diminish or reverse the fertilizing effect of added nitrogen, particularly if phosphorus currently limits plant growth (referred to hereafter as the Limitation Hypothesis). I did not expect precipitation change to have direct negative effects on soil fertility. However, if plants increased growth with precipitation change, increasing plant demand for macronutrients could diminish their concentrations in soil (Sardans & Penuelas, 2007)

Here I report the results of a year-long experiment testing the effects of simulated nitrogen deposition and dry-season precipitation increase on two dominant C-4 grass species and their associated soils, in the Brazilian Cerrado. I tested the Fertilization and Limitation Hypotheses by experimentally adding water during the dry season in amounts and intervals consistent with IPCC predictions for this area of South America (Magrin *et al.*, 2007) and adding nitrogen in amounts and composition (ammonium nitrate) relevant to future nitrogen deposition predictions (Phoenix *et al.*, 2006). As my objectives were to disentangle the direct and indirect effects of my treatments, I measured soil fertility factors in addition to plant nutrient and growth characteristics.

CHAPTER 2
METHODS

Study Site

This study was conducted at the Estação Ecológica do Panga, a 404 ha preserve 40 km from Uberlândia, Minas Gerais, Brazil (19˚10′S, 48˚23′W, Fig. 2-1). Panga contains most of the major Cerrado vegetation physiognomies (mata semidecídua, mata galeria, vereda, cerradão, cerrado sensu stricto, de Oliveira-Filho & Ratter, 2002) . The climate is subtropical, with approximately 1600 mm rainfall per year, monthly average temperatures between 20˚C and 25˚C, and an almost rainless dry season between May and September (Instituto de Geografia, 2008). The soil is a highly weathered Oxisol with a high clay content and low pH; it is classified as an Anionic Acrustoxe (Soil Survey Staff, 2003) according to US soil taxonomy, or a Latossolo Vermelho-Amarelho (EMPRAPA, 1999) in the Brazilian soil classification system. I conducted my research in a vegetation physiognomy called *campo ralo*. *Campo ralo* vegetation is typified by dense grass cover interspersed by small stature trees and shrubs (Ottmar *et al.*, 2001). The preserve is generally protected from grazing or other agricultural activities, but is subject to occasional anthropogenic fires originating on adjoining roads and farms. The most recent fire in the study area occurred in 2006, 2 years before the beginning of the experiment.

Species

The focal species for this study were two native caespitose C-4 perennial grasses, *Tristachya leiostachya* Nees and *Loudetiopsis chrysothrix* (Nees) Conert (Poaceae, Tribe: Arundinelleae, Fig. 2-2). *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. Both species flower between February

and April and are co-dominant in the *campo sujo* physiognomy where the experiment took place. Combined, they accounted for 69% of the aboveground biomass in the study area (*Loudetiopsis* =12%, *Tristachya* = 57%, all other species < 5%, E. Bruna and H. Vasconcelos, *unpublished data*). In addition to being locally common, both species have wide geographic ranges: *Tristachya* ranges from southern Brazil to Paraguay and *Loudetiopsis* is known from eastern Bolivia to southern Brazil, and Paraguay (Missouri Botanical Garden, 2009).

Experimental Design

To select grass individuals for the experiment, I first established 6 transects 50 meters apart and 150 meters (5 transects) or 50 meters (1 transect) in long in a relatively homogeneous area (e.g., similar aspect, slope, vegetation). I chose locations and species along transects by randomly generating 32 numbers from 0-149 and randomly assigning species to each location before going into the field. Approximately 32 individuals (N=16 of each species) were located along each transect, separated by a minimum of 2 meters and sometimes as much as 10 meters. In total I established 80 plots for each species within an area of approximately 150 m x 200 m during a one-week period in May 2008. With a few exceptions individuals were from intermediate size classes for each species ($18.3 \leq 36.7$ cm for *Tristachya*, $9 \leq 18$ cm for *Loudetiopsis*, based on a preliminary random sample of individuals in the study area). I then delineated plots of 50 x 50 cm around each focal individual Within each plot I clipped all above-ground biomass surrounding the focal individual; This left all roots and other below-ground biomass as well as leaf litter undisturbed. I recorded the species identity and total biomass (after drying for 48 hours at 60 degrees C) of all collected biomass. I clipped re-growth at 2-3 week intervals throughout the experiment to reduce aboveground competition.

Treatments

I randomly assigned treatments to individuals for a total of N = 20 plants per species per treatment (i.e., control, nitrogen, water, nitrogen x water). I added nitrogen in the form of ammonium nitrate (NH_4NO_3) fertilizer applied at the rate of 25 kg ha^{-1} yr^{-1} nitrogen (2.5 g m^{-1} yr^{-1} N) divided into 4 applications (June 2008, Sept. 2008, Dec. 2008, Feb. 2009) to simulate atmospheric deposition throughout the year. Previous work estimated a total N deposition rate of 9.5 kg ha^{-1} yr^{-1} N near Uberlândia (between 1997-1999), with the wet deposition component made up of 48% ammonium and 38% nitrate . Given development trends in the region, including urbanization and intensifying agricultural production (Cavalcanti & Joly, 2002) , it is unlikely that deposition rates decreased in the ten year period between the estimate and my study.

I added water in the dry season in amounts consistent with climate models that predict increasing rainfall in the Cerrado region (Lilienfein & Wilcke, 2004). These models predict that rainfall will continue to increase, following the trend observed over the last 40 years in the Cerrado (Haylock *et al.*, 2006). In my water addition I applied two liters of water over 24 hours (8 mm/day) with drip irrigation. These water addition pulses were divided by alternate dry periods of 2 and 9 days between June and August. This is about half of the average rainfall per 24 hours, on days with rain, during the wet season (October-April, 14.2 mm/day or 3.55 liters over a 50 x 50 cm plot area; data collected at field station, 2003-2004, *unpublished*). However, since dry season rainfall averages just 4.5 mm/month (May-Sept.) the addition represented a substantial rainfall increase for this part of the year (data collected at field station, 2003-2004, *unpublished*). In total I added 72 mm of water, or approximately 5% of the average annual rainfall, in addition to the ambient precipitation, 9.5 mm between June-August 2008 (data from Uberlândia, 40 km from site, 2004-2008, Instituto de Geografia, 2008).

I measured volumetric water content with Soil Moisture Smart Sensors (Onset Computer Corp., Bourne, MA, USA) in watered, control, and nitrogen treatments from July 2008 to August of 2008. In total I monitored soil moisture during 6 watering treatments with 4 or 5 sensors in different plots (varied by treatment) to confirm the efficacy of my water addition. Dataloggers recorded data every 5 minutes and were moved approximately every 10 days to new sites. Average, maximum, and minimum daily soil moisture was significantly different ($p < 0.0001$ for minimum, maximum, and average, F-value: avg.: 66.10, max.: 105.17, min.: 32.82) between watered and un-watered plots during this period (general linear model with day as a random factor). Average daily average volumetric water content (m^3/m^3) in watered plots was 6 times water content in un-watered plants during the study period.

Light Availability

Because light availability can mediate plant and soil responses to my simulated global change factors (Cruz, 1997, Ludwig *et al.*, 2001), I measured photosynthetically active radiation (PAR) with a quantum line sensor ceptometer (Accupar LP80, Decagon Devices, Pullman WA) on a cloudless day between 11am and 2pm on Mar. 3, 2009. I averaged three 80 cm measurements taken by bisecting the plot at different points at approximately the height of the tallest leaves of each individual.

Plant Response

I quantified reproductive output by measuring number of flowering tillers and number of florets per flowering tiller for all individuals before (May 2008) and after the treatments were applied (March 2009). I estimated senescence by counting all green leaves on the plant and dividing the number by genet area in August of 2008. A larger value of this index corresponded to lower leaf senescence during the dry season. Growth was quantified by measuring the

diameter around the base of each genet (individual bunchgrass) before and after the treatments (10 month period) to quantify growth. To control for the effects of original size and shading on growth, I modeled the diameter change in response to treatments with the original diameter and average PAR as covariates in the analyses. Bunch diameter to was used to quantify growth because diameter significantly correlates with total biomass for both species (*Loudetiopsis:* R^2 = 0.23, p = 0.003, *Tristachya*: R^2 = 0.25, p = 0.014).

I collected all plants at peak biomass (the end of wet season) over a 4 week period in March- April 2009. I randomized plant collections by treatment and species to avoid any systematic effects of time on biomass estimation over the collection period. I trenched around the perimeter of the plot and excavated the plant to a depth of approximately 15 cm for *Loudetiopsis* and 20 cm for *Tristachya*. I attempted to recover as much of the root biomass connected to the plant as possible. To quantify remaining root biomass, I collected five 6 cm deep cores in a line bisecting the soil remaining in the plot area after plant removal. I sieved the cores for roots with a 2 mm sieve and dried the material at 55 ˚C for a minimum of 48 hours (until reaching constant weight). Root collections may have included grass roots of other neighboring grass individuals. Remaining coarse root mass averaged 0.0015 g/cm^2 (se $\pm$ 0.0009), while average dry root mass per plant was 62.33 g (se $\pm$ 38.28) for *Loudetiopsis* and 399.69 g (se $\pm$ 255.83) for *Tristachya*.

I separated plants into dead vegetative tillers, live vegetative tillers, and flowering tillers. I also sorted all biomass into live leaves, dead leaves, flowering tillers, flowering parts (florets and seeds), and roots and dried the material at 55 ˚C for a minimum of 48 hours (until the samples reached constant weight). I calculated root:shoot ratios by dividing the total aboveground biomass, including dead leaves and reproductive parts, by the root biomass.

Soil and Foliar Nutrients

I measured plant available nitrogen by extracting ammonium and nitrate from mixed bed resin bags buried in the top 10 cm of 76 plots (32 for *Loudetiopsis*, 44 for *Tristachya*) in February – March 2009. I first charged resins with 1 M NaCl and extracted with 2 M KCl after 28 days of incubation in the plots. I also collected soil samples from the first 10 cm of the soil (March 2009), homogenized the material, and extracted 10g of field-moist soil with 2 M KCl within 24 hours of collection. We incubated 10 g of the same soil sample for 7 days at room temperature in the lab and extracted with 2 M KCl to quantify mineralization rates. We measured gravimetric water content in subsamples of field-moist soil by drying them for 48 hours at 110˚C. Soil and resin extracts were analyzed colorimetrically with an Astoria Autoanalyzer (Astoria-Pacific, Inc. Clackamas, OR, USA) for nitrate and ammonium concentration. Soil nitrogen values were adjusted for bulk density and gravimetric soil moisture.

I dried separate soil samples at 55˚ C for 48 hours for pH and macronutrient analysis. pH was measured in deionized water (ratio: 1:2.5 soil:H_2O). I extracted potassium and phosphorous with Mehlich (HCl-H_2SO_4) solution (K ratio: sample:solution 10:1, P sample:solution 20:1) and analyzed nutrient concentrations with flame emission spectrometry for potassium (B462, Micronal, São Paulo, SP, Brazil) and atomic absorption spectrometry for phosphorus (Cary 50 Conc UV-Vis, Varian Inc., Palo Alto, CA, USA). I used 1 M KCl to extract aluminum, calcium, and magnesium (Ca & Mg ratio sample:solution 100:1, Al ratio: sample:solution 10:1). I analyzed aluminum content by titration with NaOH in the presence of bromothymol blue. I analyzed calcium and magnesium with flame emission spectrometry (GBC Scientific Equipment 932 A, Dandenong, VIC, Australia).

I washed a subsample of green undamaged leaves from each individual with deionized water, dried the material for 48 hours at 60˚ C, and ground the samples in a plant mill (Marconi Equipamentos, MA 048, Piricicaba, SP, Brazil). I digested tissue samples with the Kjeldahl method, steam distilled the digest into boric acid, and titrated with sulfuric acid. For foliar phosphorus, I digested tissue in nitric and perchloric acid and analyzed solution P concentration with atomic absorption spectrometry (Cary 50 Conc UV-Vis, Varian Inc., Palo Alto, CA, USA).

Statistical Analysis

Reproductive Variables

Reproductive output variables were analyzed separately for each species. I analyzed the likelihood an individual would flower with my treatments with a binomial model. The response variable was flowering or non-flowering state while nitrogen and water were fixed effects. I conducted the same analysis in a separate regression model, with resin available nitrate and ammonium as fixed effects, to evaluate the direct effects of nitrogen on flowering. I analyzed treatment effects on the number of flowering tillers per flowering individual and number of total spikelets with a negative binomial generalized linear model with nitrogen and water as fixed effects. I used a general linear model to evaluate treatment effects on number of spikelets per tiller.

Growth and Foliar Nutrient Variables

I calculated the difference in diameter between year one and year two and analyzed the effects of the treatments with a general linear model with nitrogen, water, original diameter, and average PAR as fixed effects. I analyzed treatment effects on senescence (number of green leaves by area) with an ANCOVA with original diameter as the continuous variable.

To explore the effects of treatments on root:shoot ratio, I evaluated the response of root:shoot ratios treatments with a gamma distribution in a generalized linear model. I used the gamma distribution because goodness-of-fit tests and analysis of residual deviance showed that it improved model fit over the normal distribution and models with transformed variables. I analyzed the effects of my treatments on total aboveground biomass, total dead leaf biomass, and total live leaf biomass with general linear models. I evaluated change in foliar nitrogen and phosphorus concentrations and nitrogen: phosphorus (N:P) ratios to treatments with an ANCOVA with live leaf mass as the continuous covariate. All plant response variables were analyzed with separate models for each species.

Soil Variables

I tested the effects of treatments and species on pH, nitrogen, potassium, calcium, and aluminum concentrations with general linear models with nitrogen, water, and species as fixed effects. I used a generalized linear model with the gamma distribution to evaluate treatment effects and species effects on soil phosphorus, resin available ammonium, and nitrate and ammonium mineralization rates. Because species significantly interacted with the water treatment effects on soil phosphorus, I included that interaction into the model. I tested treatment effects on resin available nitrate, soil ammonium and nitrate, and nitrate and ammonium mineralization rates with a general linear model.

Model Assumptions and Variable Transformations

I tested for homogeneity of variance with Levene's test and applied log transformations when necessary to meet the assumption of normally distributed residuals for general linear models. For all transformations and non-normal distributions (gamma, binomial, and negative binomial) I reported back-transformed means and standard errors or 95% confidence intervals. I

used Dunnett's test for comparison of treatment means to control values. All analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

CHAPTER 3
RESULTS

Reproductive Response

There was no statistically significant nitrogen treatment or water treatment effect on the likelihood that an individual would flower with nitrogen or water for *Loudetiopsis*. However, there was a significant positive interactive effect of the combination of nitrogen and water on plant flowering for this species (95% of individuals flowered with nitrogen x water treatment, 89% for nitrogen, and 65% for water, Table 3-1, Fig. 3-1). However, when these values were compared to controls, these differences were not significant (Dunnett's test, Table 3-1). With *Tristachya,* water alone increased the percentage of flowering *Tristachya* individuals from 45% to 70% (Table 3-1, Fig. 3-1). Contrary to expectations, higher resin available ammonium and nitrate values decreased the likelihood that a *Tristachya* individual would flower. There was no significant effect of resin available nitrogen on the flowering likelihood for *Loudetiopsis* (Table 3-2).

While only the interaction of water and nitrogen treatments increased the likelihood that an individual *Loudetiopsis* would flower, those individuals that did flower produced significantly more flowering tillers in response to both water alone and the combined water and nitrogen treatment. Individuals of all three treatments produced significantly more flowering tillers than controls (Table 3-3, Fig. 3-2). Flowering *Tristachya* individuals did not produce more flowering tillers with treatments or in contrast to control values (Table 3-3, Fig. 3-2).

For the plants that did flower, I tested whether the nitrogen and water treatments affected reproductive output - defined as the total number of spikelets produced. The water treatment for *Loudetiopsis* was the only species and treatment combination with a significant effect on total spikelet number. However, the total number of spikelets for *Loudetiopsis* for watered plants

(mean=57.15) and plants receiving the nitrogen and water treatment (mean=60.31) was significantly greater when compared to control plant values (Dunnett's test Water: p=0.09, Nitrogen: p=0.03, Table 3-4). None of the treatments were significant for *Tristachya* (Table 3-4).

The number of spikelets per flowering tiller marginally increased with nitrogen addition for *Loudetiopsis*. There was no significant change in number of spikelets per tiller with either water or the water and nitrogen treatment, in contrast to this species' response to water with other reproductive variables. There was no significant difference in spikelets per flowering tiller with treatments for *Tristachya* (Table 3-5).

Growth, Allocation, and Foliar Nutrients

Tristachya bunchgrass diameter increased significantly while *Loudetiopsis* diameter growth decreased with added nitrogen (Table 3-6, Fig. 3-3). For both species the larger the individual (the greater the original diameter) the lower the growth rate (decreased diameter difference). Greater photosynthetic active radiation (PAR), or less shading, correlated with higher growth rate for both species (Table 3-6). Both water and nitrogen significantly reduced root:shoot ratios for *Loudetiopsis*, but only plants in the combined treatment (water x nitrogen) had significantly lower ratios than control plants (Table 3-7). There was no significant difference with any treatment for *Tristachya*.

The significant decrease of *Loudetiopsis* root:shoot ratios with water and nitrogen seemed to suggest increased aboveground growth while the decreasing effects of nitrogen on diameter suggested lower growth rates. To test for treatment effects on the general relationship between aboveground biomass and diameter with this species, I analyzed the effects of treatments on the amount of aboveground biomass by diameter, the amount of dead biomass by diameter, and the amount of live leaf biomass by diameter (Table 3-8). *Loudetiopsis* had significantly more

aboveground biomass by diameter (p=0.03) with the water treatment. This was probably due to the significant increase in dead aboveground material by diameter (p=0.01) with water addition. In other words, the lower diameter growth rate for *Loudetiopsis* with the water treatment was combined with a significant decrease in root:shoot ratio with water addition due to increased dead aboveground biomass with the water treatment. There were no significant treatment effects on biomass by diameter for *Tristachya*.

Loudetiopsis individuals had significantly more green leaves per bunch area by August of the dry season with the water treatment (Table 3-9). Because of the water effect on dead aboveground biomass for *Loudetiopsis* I tested whether or not the number of senesced leaves might correlate with the dead aboveground biomass/diameter variable. There was a significant positive linear relationship (p=0.04, F-value = 4.34, $R^2 = 0.05$) between the number of green leaves per area and the amount of dead biomass at the end of the experiment with *Loudetiopsis*, but no hint of a significant relationship for *Tristachya* (p = 0.99, F-value <0.01, $R^2 = \leq 0.01$). None of the treatments had significant effects on number of green leaves per area for *Tristachya*. With increasing diameter both species had significantly fewer green leaves per area (Table 3-9).

Nitrogen concentration and N:P ratios for *Loudetiopsis* decreased with increasing live leaf biomass (which was not significantly affected by treatments) (Table 3-10 & Table 3-12). *Tristachya* nutrient concentrations did not vary significantly with live leaf biomass. Treatments did not significantly affect nitrogen concentration for either species, contrary to my prediction that nitrogen addition would increase foliar nitrogen content (Table 3-10, Fig. 3-4). Phosphorus concentration marginally decreased with nitrogen addition for *Loudetiopsis* when compared with control values. However, phosphorus concentrations increased with both nitrogen and water treatments for *Tristachya*, and their combination resulted in a significant difference compared to

controls (Table 3-11, Fig. 3-5). Nitrogen addition significantly increased nitrogen: phosphorus ratios (N:P) for *Loudetiopsis* (Table 3-12). *Tristachya* N:P ratios with the combined nitrogen and water treatment were significantly lower than control values (Dunnett's test, Table 3-12). Water, rather than nitrogen, or an interactive effect, appeared to be driving this result, as it was the only factor significant in the model (Table 3-12).

Soil Response

Plant-available soil phosphorous significantly increased with nitrogen addition and differed by species (Table 3-13). The water treatment significantly affected phosphorus concentrations for both species, but these effects were in opposite directions (Table 3-14). In *Loudetiopsis* plots, water addition lowered soil phosphorous concentrations by about 10% ($p=0.07$, Table 3-14, Fig. 3-6). However, *Loudetiopsis* plots with nitrogen addition marginally increased phosphorous concentrations (Table 3-14, Fig. 3-6). For *Tristachya* nitrogen and combined water and nitrogen treatments significantly increased soil phosphorous concentrations, but only the combined nitrogen and water values significantly differed from controls (Dunnett's contrasts, $p = 0.03$, Table 3-14).

Though I expected that nitrogen addition might change pH or aluminum mobility, my data did not support this hypothesis. However, potassium availability marginally decreased and calcium marginally increased with nitrogen addition. None of the soil factors changed with water addition, and there were no significant differences for pH, Al, Ca, or K between control and treatment values (Table 3-15). Resin extracted nitrate and ammonium concentrations significantly increased with water addition, but not with nitrogen addition. Species did not significantly affect either resin available nitrogen form (Table 3-16, ammonium, Table 3-17, nitrate). The mineralization rate for nitrate and ammonium did not vary by treatment or species

(Table 3-18 ammonium, Table 3-19 nitrate). Soil ammonium did not vary by treatment or species, but soil nitrate marginally decreased with water addition (Table 3-20 ammonium, Table 3-21, nitrate).

CHAPTER 4
DISCUSSION

This research demonstrates that global change factors have the potential to alter growth and reproduction of two co-dominant Cerrado bunchgrasses, and that these effects vary by species (Table 4-1). As both focal species are C-4 grasses, the divergence in species response I observed is not due to known functional group differences in nutrient demand (Craine *et al.*, 2002). The data did not uniformly support the original expectation that added water and nitrogen would result in increased vegetative and reproductive biomass.

Reproductive Response

In general, *Tristachya* reproductive variables were unresponsive to treatments, whereas *Loudetiopsis* altered a suite of flowering traits in response to water and nitrogen addition. Contrary to the fertilization hypothesis, increased nitrate and ammonium were not correlated with flowering of *Loudetiopsis* and decreased flowering of *Tristachya.* While the experiment was not designed to determine how ammonium and nitrate levels directly affect plant phenology, the data suggest that additional nitrate and ammonium will not necessarily increase grass flowering as they do in some temperate grasslands (Silletti *et al.*, 2004). Though the results suggest that the combination of nitrogen deposition and precipitation could increase flowering with these two species, the lack of positive correlation with resin-available nitrogen concentration does not confirm a fertilization effect of nitrogen addition. However, since water addition was associated with increased resin available nitrogen, water addition might have increased flowering by increasing microbial nitrogen mineralization.

Conclusions based on these results are qualified by the large reduction in flowering between year one (before the fertilization began) and year two of the study for all plants, including controls. In the reproductive season prior to the experiment (Feb. – March. 2008) 94 %

of *Tristachya* individuals flowered, while only 58% flowered in 2009. *Loudetiopsis* phenology followed the same pattern, diminishing from 83 % to 58% flowering individuals between 2008 and 2009. This effect was not due to the experimental setup; plants in the study area outside of the experiment were as likely to flower and had on average the same numbers of flowering tillers as control plants. This highly significant difference in years suggests that both species are capable of higher flowering rates then recorded during the experiment. It is possible that a fire that in the study area in 2006 is related to the higher reproduction I observed in 2008. South American savanna grass species as a group tend to respond to fire by increasing flowering, though dependence on fire for reproduction varies by species (Baruch & Bilbao, 1999, Sarmiento, 1992). If so, my results show that variability in annual climate and nitrogen deposition can increase reproductive output, but may pale in comparison to the effects of other environmental changes like fire.

Growth Response

While both water and nitrogen affected the species' reproductive output, only nitrogen significantly affected diameter growth. However, the nitrogen addition had opposite effects on the two species. *Loudetiopsis* grew less with the simulated nitrogen deposition treatment, whereas *Tristachya* grew more, a response consistent with nitrogen induced tiller production for bunchgrasses in general (Tomlinson & O'Connor, 2004). Water did not seem to increase genet diameter growth, contrary to predictions. The result is especially puzzling with *Loudetiopsis* because water decreased the root:shoot ratio and reduced leaf senescence. *Loudetiopsis* genets increased aboveground biomass by diameter with water, but the increase was primarily due to an increase in dead tissue. This response correlated with this species' decrease in senescence with water addition. It is possible that the additional water resulted in either decreased leaf life-span or

increased leaf production during the dry season, which did not translate into increased live biomass or diameter by the end of the study.

The responses I observed diverge from results in to similar experiments in other ecosystems. Research focusing on annual grass species in California found that more grass species responded to nitrogen than precipitation change (Zavaleta *et al.*, 2003). While biomass generally increased with water addition, nitrogen led to even higher values, and the combined water and nitrogen treatment led to the highest biomass accumulation (Zavaleta *et al.*, 2003). In this experiment, nitrogen affected both species, but only one species increased growth (*Tristachya*) whereas the other increased reproduction (*Loudetiopsis*). The species that decreased growth with nitrogen (*Loudetiopsis*) increased dead biomass accumulation instead. Finally, I did not observe any significant interactive effects of nitrogen and water on growth variables for either species.

Leaf Senescence

Species drought tolerance traits probably influenced the differential leaf senescence response to water addition in the study. Even in control plants, the two species differed in senescence patterns: *Tristachya* maintained a higher density of green leaves in the dry season than *Loudetiopsis* in control plants (*Copeland,* unpublished data). Neither species completely senesced in the dry season, a typical behavior for Neotropical perennial savanna grasses (Sarmiento, 1992). The species differences in senescence may be related to root characteristics, as *Tristachya* had deeper and larger roots than *Loudetiopsis.* The complementary nature of root structure between these two co-dominant grass species mirrors a pattern found elsewhere (Fargione & Tilman, 2005), that allows co-occurring perennial grasses to exploit different nutrient and water sources.

A comparable set of experiments also found dramatic differences in species' growth response between the responses of two co-dominant perennial C-4 grasses in the Great Plains to altered precipitation (Silletti & Knapp, 2001, Silletti *et al.*, 2004). They identified a suite of traits, including decreased leaf senescence and higher root:shoot ratio, which conferred greater drought resistance on the dominant species (*Andropogon gerardii*) (Swemmer *et al.*, 2006). These same traits may explain the lack of response of *Tristachya* to water addition, as this species also demonstrated lower leaf senescence in the dry season and higher root:shoot ratio.

Foliar Nitrogen and Phosphorus

Foliar nutrient concentrations changed in response to the treatments, but these responses differed by species. Both species had generally low nitrogen: phosphorus ratios (control means *Loudetiopsis* : 9.43 (± 1.16), *Tristachya* : 9.74 (± 1.17)), which could indicate nitrogen limitation (Tessier & Raynal, 2003). However, recent examinations of N:P ratios in tropical forests (Townsend *et al.*, 2007) and grasslands (Craine *et al.*, 2008) suggest that N:P ratios may not predict absolute limitation. In one study in as early successional tropical forest, tree species had higher nitrogen: phosphorus ratios (13:1 and 15:1) then the grass species (9:1) yet the tree species increased both biomass and foliar nitrogen concentrations with added nitrogen, whereas neither factor increased with the grass species (Davidson *et al.*, 2004). In this experiment, one species, *Loudetiopsis*, had significantly higher N:P ratios with nitrogen whereas *Tristachya* had lower N:P ratios in the presence of water. The measurements of phosphorus and nitrogen concentrations in response to treatments suggest that foliar phosphorus, rather than nitrogen, drove N:P changes in both species

I had predicted that foliar nitrogen, not phosphorus, would change in response to my treatments, via the direct effects of nitrogen fertilization on plant available nitrogen or indirect

stimulation of microbial nitrogen mineralization with dry season water addition. What might explain change in foliar phosphorus in lieu of nitrogen? Plant N:P ratios vary based on a wide variety of characteristics, including functional group. It may be that Neotropical grass species in general simply maintain lower leaf N:P ratios relative to other savanna plant species (such as trees), a suggestion borne out by data from a similar study in the neotropics (Davidson *et al.*, 2004). The same pattern may exist in the preserve where this study took place. The average nitrogen concentration for 93 tree species from Estação Ecológica do Panga was 25 g kg^{-1} with phosphorus concentrations of 2 g kg^{-1} on average (Hardisan, 2005). The tree species average N:P ratio of 12.5:1 is higher than both of the grass species ratios (8.5 – 10.5 N:P), suggesting that the C-4 grass species N:P ratios may be lower than those of other functional groups in the study area.

Species differences in foliar nutrient changes in response to treatments could be due to a variety of species traits other than functional group identity. While my data are insufficient to explore all possible characteristics that could be responsible for differences in foliar chemistry, they do suggest do suggest some traits correlated with the foliar responses to treatments. *Loudetiopsis* increased N:P ratios with increasing live leaf biomass, suggesting nitrogen limitation, whereas the significant downward trend in N:P ratios with increasing live leaf tissue suggest that the species was capable of diluting nitrogen in leaf tissue with increased living biomass. The significant decrease in N:P for *Tristachya* is harder to explain since the decrease was not associated with any of the species few growth and reproductive responses to water and nitrogen. Another grassland study observed that water addition simultaneously increased foliar phosphorus while reducing phosphatase activity (Menge & Field, 2007) and suggested that water might stimulate phosphorus mineralization in tandem with increased nitrogen mineralization. This mechanism still does not explain the differences between species in this experiment,

however some combination of plant capacity to increase phosphotase activity with nitrogen treatments or increased uptake of mineralized phosphorus could have led to the increased foliar phosphorus concentrations in *Tristachya.* Since neither species significantly increased foliar nitrogen, the experiment did not support the proposed nitrogen fertilization effect with nitrogen deposition and increased precipitation. My alternative limitation hypothesis, that nitrogen could decrease growth by decreasing phosphorus availability, was not supported either, as one species decreased phosphorus concentrations with increasing live leaf biomass, whereas the other increased foliar phosphorus concentration.

Soils Response

The suggestion that the two perennial grass species differed in their phosphorus use and uptake is consistent with the soils data. Phosphorous increased with water in soil under *Tristachya* and decreased in soil under *Loudetiopsis*. Irrespective of species, soil phosphorus concentrations increased with added nitrogen. *Tristachya* maintained higher soil phosphorus concentrations than *Loudetiopsis* for all treatments including controls. This evidence, combined with the foliar data, suggest that *Loudetiopsis* may be limited by phosphorus when water and water and nitrogen are added.

I did not expect significant increases in soil phosphorus with added water or the divergence in species response with either the Fertilization or Limitation Hypothesis. Even though water addition decreased *Loudetiopsis* soil phosphorus, the decrease was only back to control levels. This result did not support my Limitation Hypothesis, which predicted that phosphorus and other macronutrients would decrease with added nitrogen and remain unchanged with added water.

The only significant change in resin available nitrogen forms, both ammonium and nitrogen, was the increase in resin nitrate and the decrease in soil nitrate with added water. The

increase with water could be due to increased mineralization with added water during the dry season, as observed in other savanna ecosystems (Augustine & McNaughton, 2004). It is possible that the decrease in soil nitrate is related to increased plant uptake of soil nitrogen or leaching. In aggregate, the increase in total available nitrate for 28 days with a decrease in available nitrogen at one time point suggests increased nitrate turnover in soil with added water. Though I had predicted that soil nitrate and ammonium would increase with added nitrogen, but the results do not support this expectation. It is possible that plant uptake, soil immobilization, gaseous losses, or some other mechanism explain the lack of significant change in available nitrogen with fertilization.

Other soil nutrient factors did not seem as sensitive to simulated global change factors as phosphorus and nitrogen. As predicted in the Limitation Hypothesis, pH decreased and extractable aluminum increased with added nitrogen, however these differences were not significant. Calcium increased marginally and potassium decreased marginally with my simulated nitrogen deposition. These changes do not particularly support the theory of general cation depletion predicted for phosphorus limited tropical ecosystems (Matson *et al.*, 1999). The fact that I observed changes in pH, Al, and cation concentrations in the extremely short time period of this study suggests that significant change in these soil factors with chronic nitrogen deposition is possible. However, there was no evidence that soil macronutrient changes resulted in decreased plant productivity. The decreased soil pH, increased Al, increased Ca, and decreased K, did not translate into decreased plant growth or allocation in general, though *Loudetiopsis* did grow less with added nitrogen. That fact that phosphorus did not decrease along with pH, as predicted, might explain the lack of support for my Limitation Hypothesis for plant growth and allocation.

CHAPTER 5
CONCLUSION

The results from this experiment indicate that nitrogen deposition and precipitation change have the potential to alter the growth and reproduction of two co-dominant grass species, and via plant effects on soils, available phosphorus and nitrogen. Since this experiment was conducted with mature native individuals at realistic levels of nitrogen and water addition, the results should accurately represent the short-term effects of these global changes on native species. This evidence also suggests the possibility of future cation depletion and increasing acidity, both factors which could eventually limit plant growth and reproduction.

Most of the species responses occurred with precipitation change rather than nitrogen addition, but this outcome could have been affected by the high levels of nitrogen deposition at the study site (Lilienfein & Wilcke, 2004). The increase of one species' N:P ratio following nitrogen addition suggests that at least one species is still limited by nitrogen. Soil phosphorus concentration also seems to be related to water addition under the influence of one of the grass species, suggesting that plant phosphorus acquisition could be affected by precipitation change.

The paradigm that tropical species on highly weathered soils are primarily limited by phosphorus (Vitousek, 1984) cannot be proven or disproven with this experiment, but it is suggestive that the responses observed were in foliar phosphorus rather than nitrogen, yet growth response to nitrogen addition was also recorded. This corresponds with a body of research that qualifies Neotropical ecosystem level nutrient limitation by phosphorus or nitrogen by factors like successional stage (Davidson *et al.*, 2004) and fire (Nardoto *et al.*, 2006). The similarities between water treatment responses of C-4 grass species here and C-4 grass species in the temperate zone (Fargione & Tilman, 2005, Fay *et al.*, 2003, Silletti *et al.*, 2004) suggest that species drought tolerance traits may determine response to altered precipitation. However, in

general, the responses to treatments we observed for foliar and growth variables did not greatly resemble biomass and flowering responses observed in temperate global change grassland experiments (Siemann *et al.*, 2007, Silletti & Knapp, 2001, Zavaleta, 2002). Water and nitrogen drove different plant and soil responses. Their combination had simple additive effects on some variables, and interactive effects on others. Overall my results imply that we cannot predict the responses of species to global change if the effects of nitrogen deposition and precipitation change are addressed independently.

The abbreviated nature of this study suggests a number of avenues for future research. Future global change research in Neotropical savannas should address the interactive effects of fire and herbivory on water and nitrogen deposition responses, given their known impact on nitrogen cycling and productivity. The relationship of both the water and nitrogen cycles to the phosphorus cycles needs to be examined, particularly from the standpoint of Cerrado plant species, which may have differential responses to global change driven phosphorus limitation.

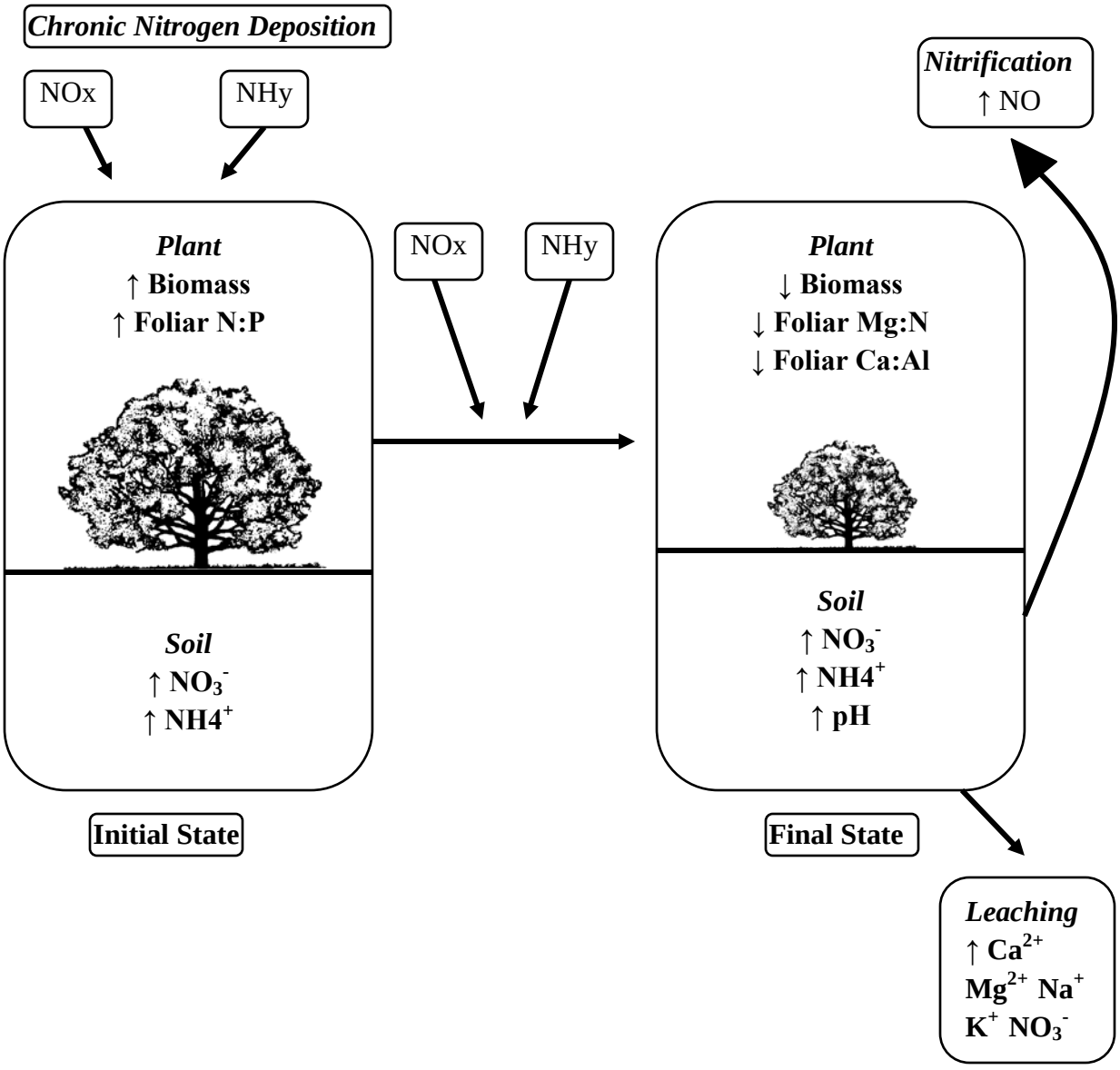

Figure 1-1. Nitrogen deposition responses in nitrogen limited ecosystems (adapted from Aber et al., 1998).

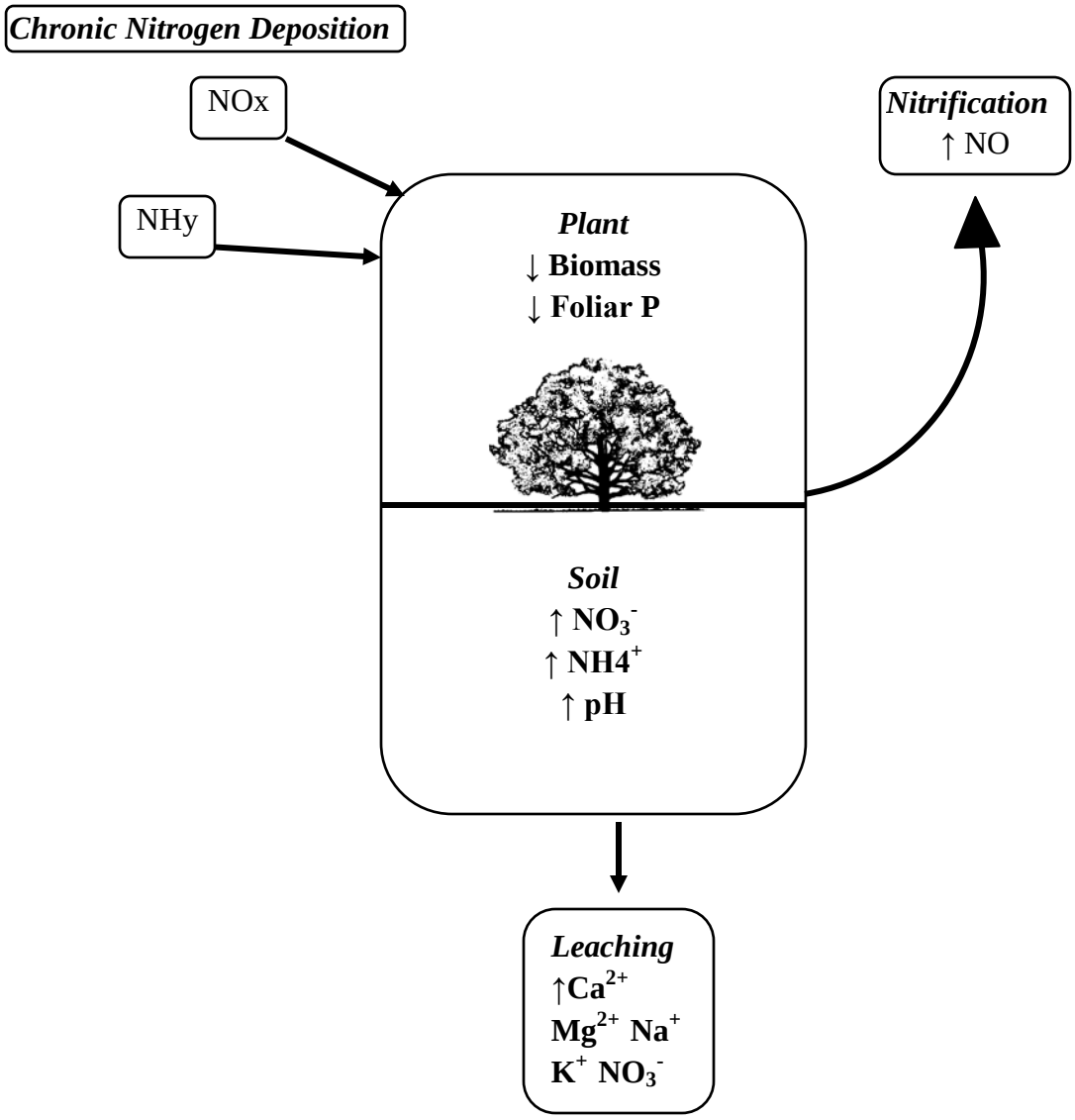

Figure 1-2. Nitrogen deposition responses in phosphorus limited ecosystems (adapted from Matson *et al.*, 1999).

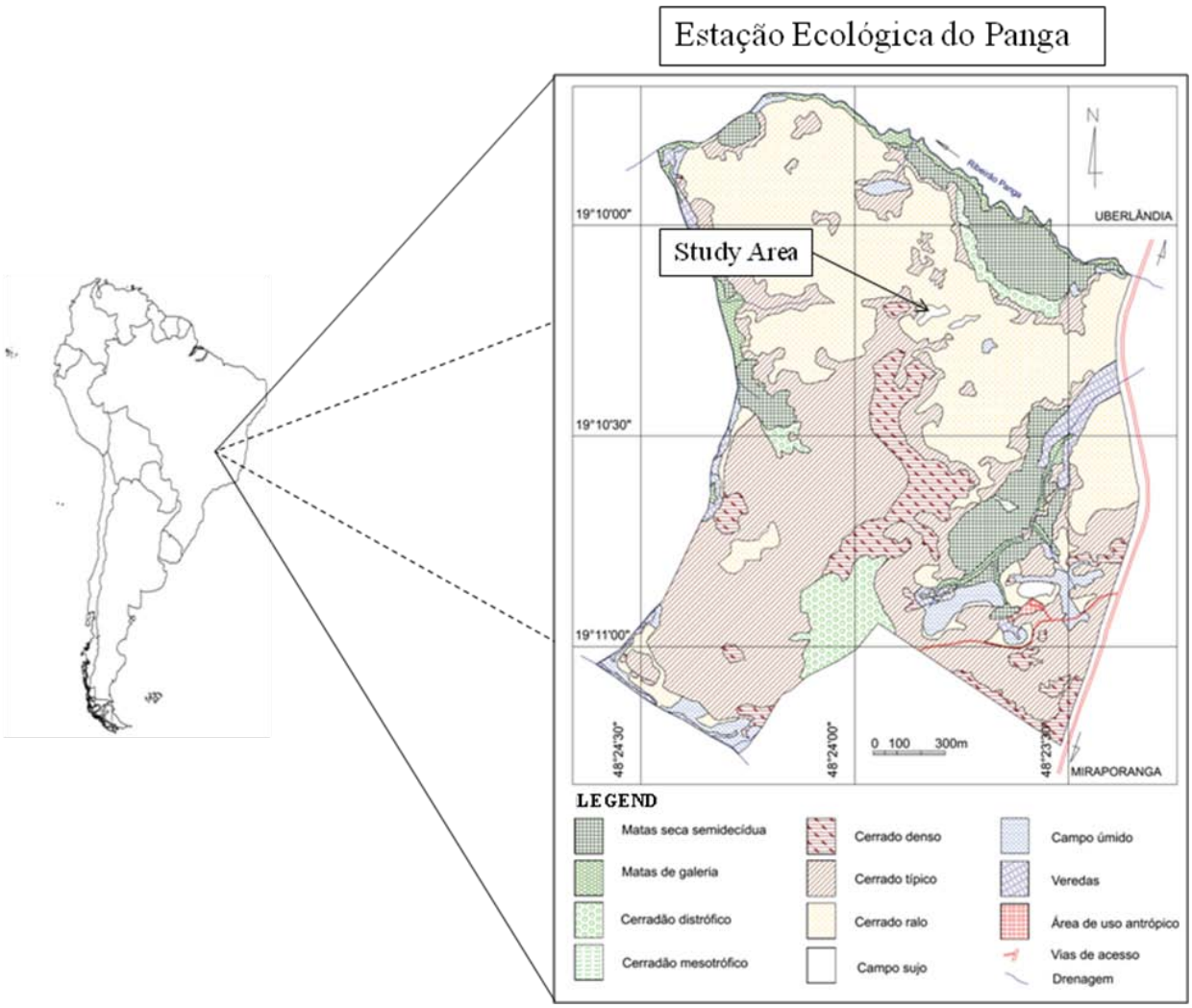

Figure 2-1. Map of Estação Ecológica do Panga and location of the study area. Legend vegetation physiognomies refer to standard vegetation classifications in Cerrado literature without English translation (Oliveira & Marquis, 2002, Ottmar *et al.*, 2001).

A)

B)

C)

Figure 2-2. Photos of focal species and habitat A) *Tristachya leiostachya* B) *Loudetiopsis chrysothrix* C) Species habitat, *campo sujo* vegetation physiognomy near plots.

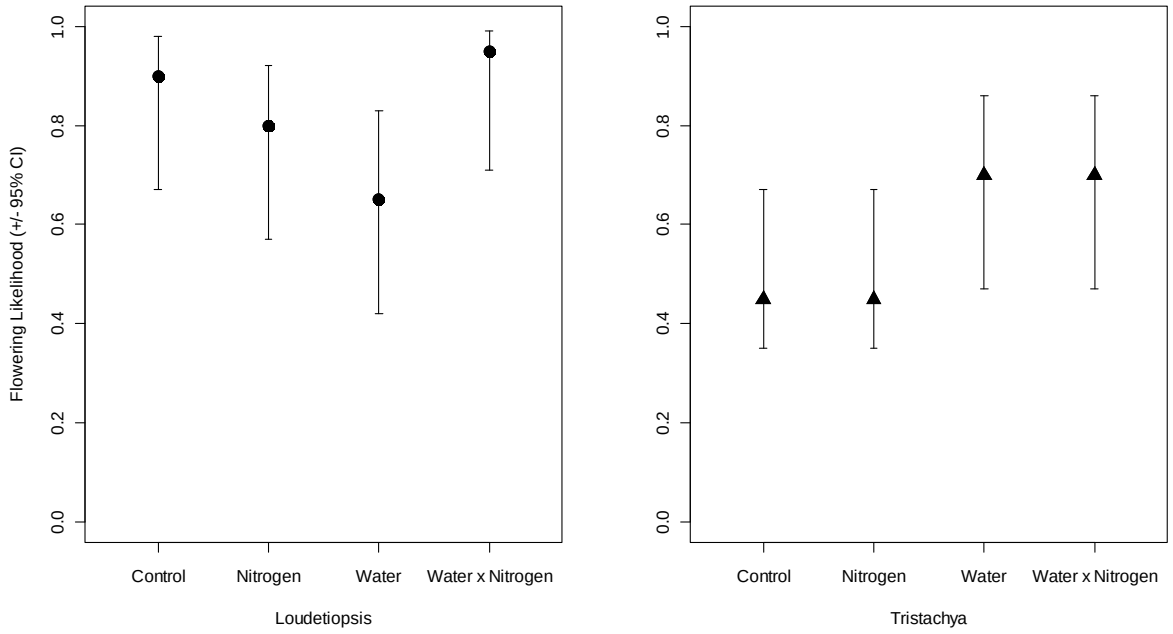

Figure 3-1. Flowering likelihood by treatment for *Loudetiopsis* and *Tristachya*. Bars represent 95% confidence intervals.

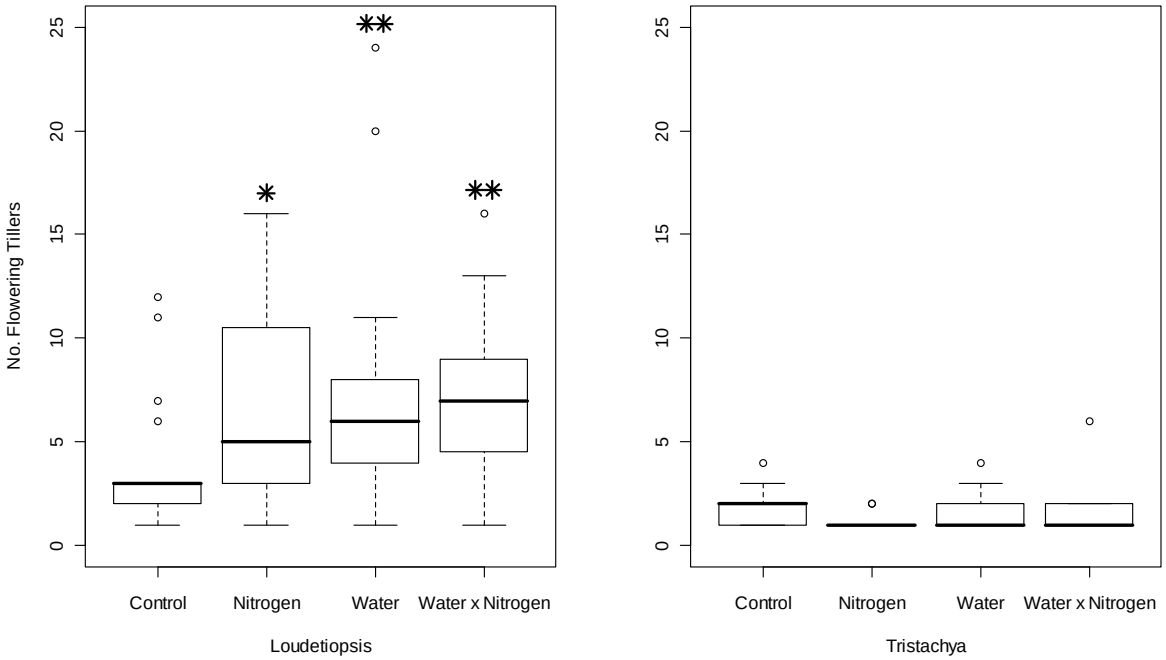

Figure 3-2. Number of flowering tillers by treatment for *Loudetiopsis* and *Tristachya*. Significant differences from controls (Dunnett's test, Table 3-3) are indicated by * for $p < 0.10$, ** for $p < 0.05$.

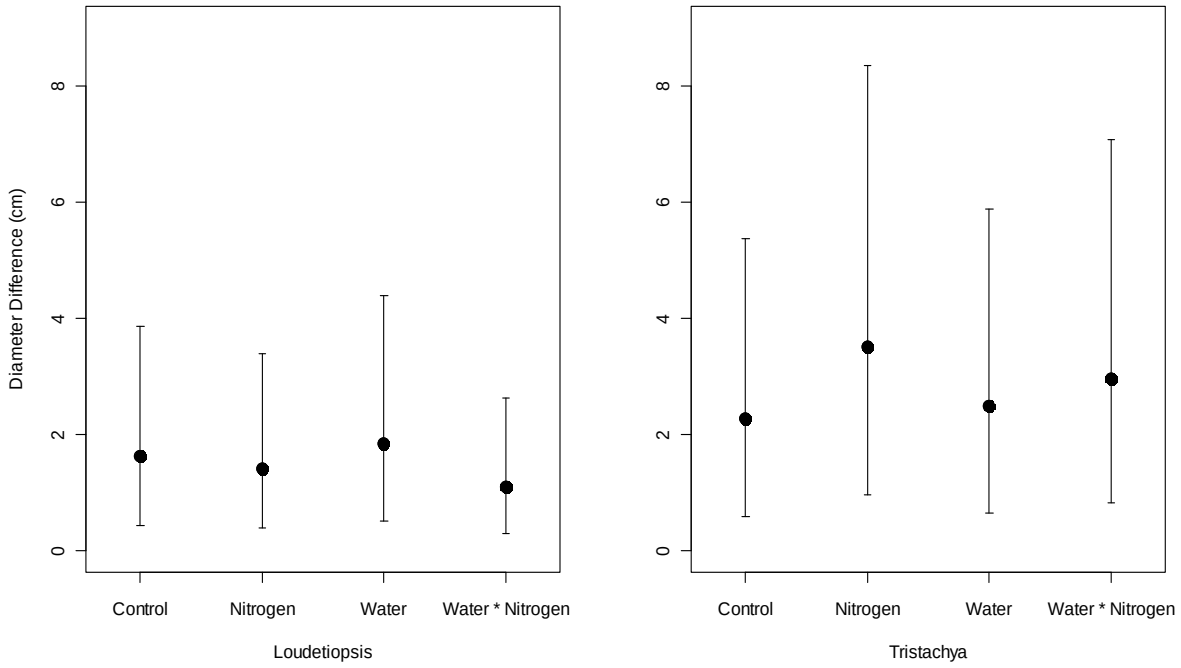

Figure 3-3. Diameter difference by treatment for *Loudetiopsis* and *Tristachya*. No significant differences between controls and treatments for either species. Reported values are adjusted by covariates in model (Avg. PAR & original diameter).

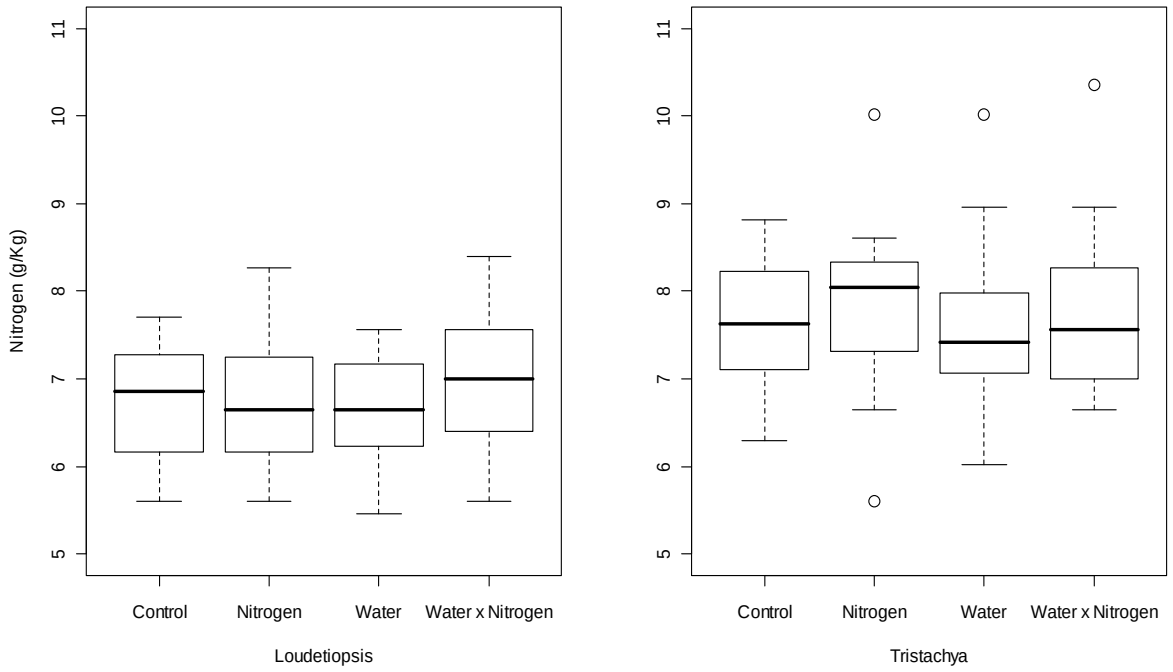

Figure 3-4. Foliar nitrogen by treatment for *Loudetiopsis* and *Tristachya*. No significant differences between treatments for either species. Reported values are raw values - not adjusted by live leaf biomass (covariate in ANCOVA model).

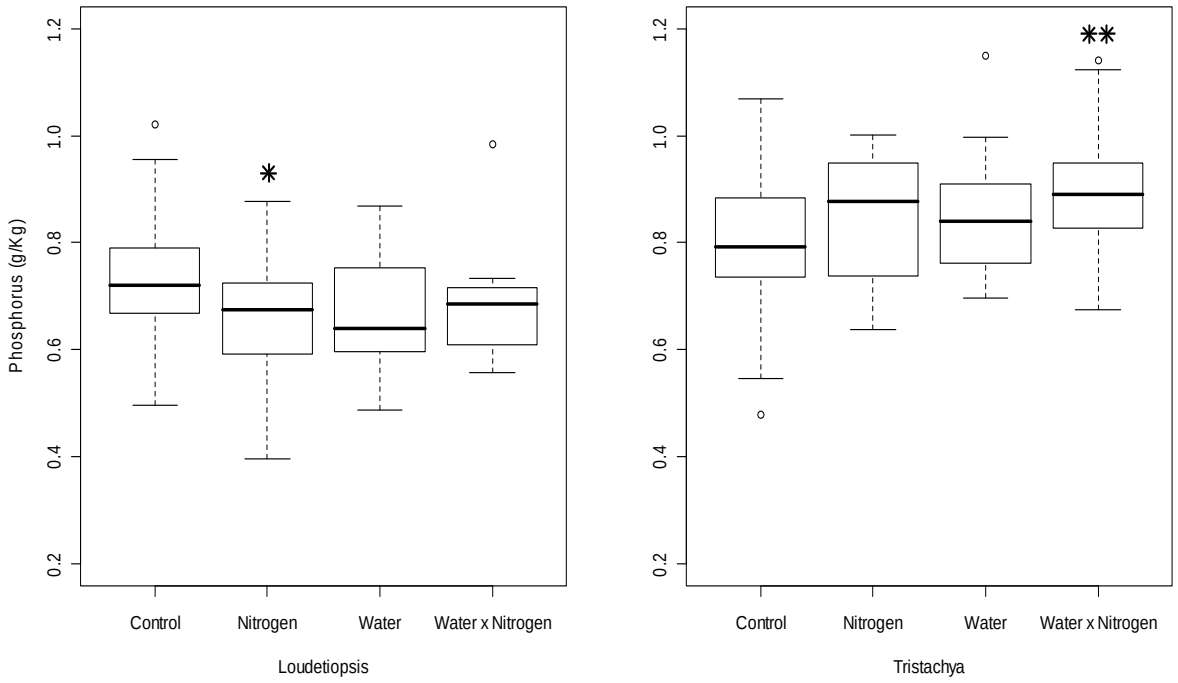

Figure 3-5. Foliar phosphorus by treatment for *Loudetiopsis* and *Tristachya*. Significant differences from controls (Dunnett's test, Table 3-11) are indicated by * for $p < 0.10$, ** for $p < 0.05$. Reported values are raw values not adjusted for live leaf biomass (covariate in ANCOVA model).

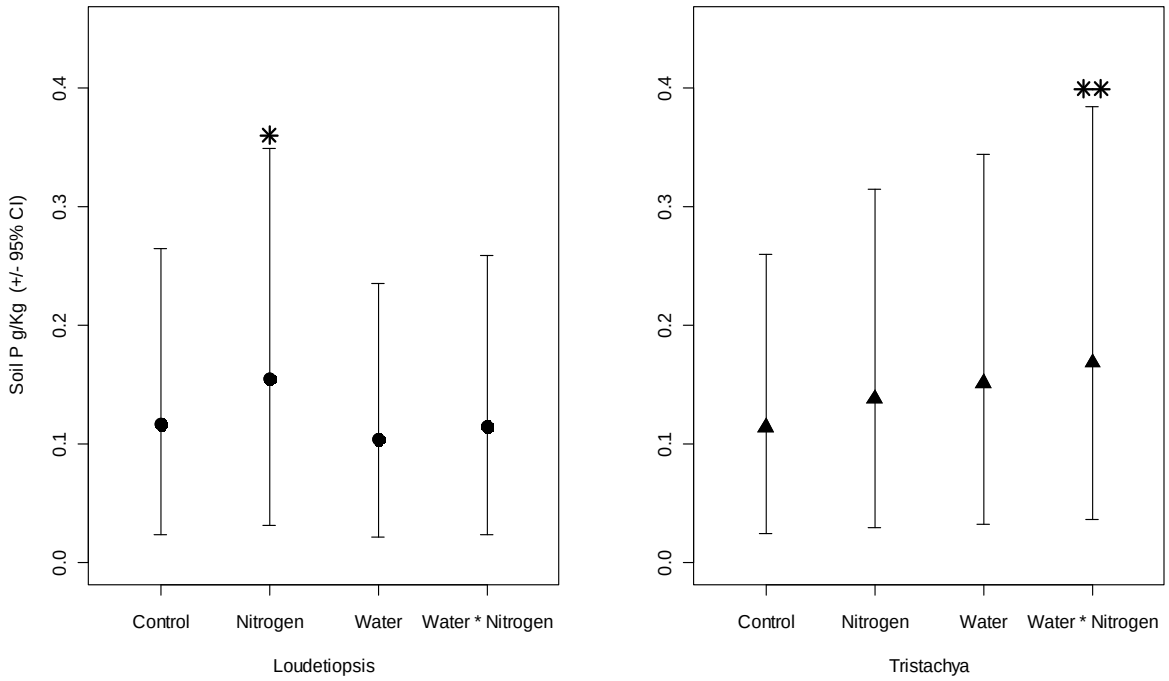

Figure 3-6. Soil phosphorus (g/Kg) by treatment for *Loudetiopsis* and *Tristachya*. Significant differences from controls (Dunnett's test, Table 3-14) are indicated by * for $p < 0.10$, ** for $p < 0.05$. Bars represent 95% confidence intervals.

Table 3-1. Flowering probability in response to water and nitrogen by species. Significance values are from logistic model with two fixed effects, nitrogen and water, and the interaction. Means are probabilities of flowering, for plots with nitrogen or water, and plots with water and nitrogen. Model fit: AIC = 76.20, n = 80 (*Loudetiopsis*); AIC = 111.92, n = 79 (*Tristachya*). Arrows indicate direction of difference from control values.

Species	Treatment	df	Wald χ^2	p	Lower Confidence Interval (95%)	Mean (Probability)	Upper Confidence Interval (95%)	Dunnett's test
Loudetiopsis	Nitrogen	1	1.0715	0.30	0.73	0.89 ↓	0.97	0.70
	Water	1	0.0002	0.99	0.66	0.86 ↓	0.95	0.18
	Nitrogen x Water	1	4.5955	0.03	0.71	0.95 ↑	0.99	0.87
Tristachya	Nitrogen	1	0.000	1.00	0.42	0.58 ↑	0.73	1.00
	Water	1	4.988	0.03	0.54	0.70 ↑	0.82	0.28
	Nitrogen x Water	1	0.000	1.00	0.47	0.70 ↑	0.86	0.28

Table 3-2. Flowering likelihood in response to resin available nitrate and ammonium by species. Significance values are from a logistic model with resin nitrate and resin ammonium as fixed effects, without interaction. Model fit: AIC = 29.74, n = 32 (*Loudetiopsis*); AIC = 61.53, n = 44 (*Tristachya*). Arrows indicate direction of effect on flowering probability.

Species	Treatment	df	Wald χ^2	p
Loudetiopsis	Resin Nitrate	1	0.1840	0.67 ↑
	Resin Ammonium	1	0.7637	0.38 ↓
Tristachya	Resin Nitrate	1	2.5481	0.11 ↓
	Resin Ammonium	1	5.436	0.02 ↓

Table 3-3. Number of flowering tillers per flowering individual by treatment and species. Significance values are from a generalized linear model with negative binomial response variable distribution with nitrogen, water, and their interaction as fixed effects. Means are probabilities of flowering, for plots with nitrogen or water , and plots with water and nitrogen. Model fit: AIC = 364.49, χ^2 = 69.12 /df = 1.11, n = 66 (*Loudetiopsis*); AIC = 137.02, χ^2 = 27.12 /df = 0.65, n = 46 (*Tristachya*). Arrows indicate direction of difference from control values.

Species	Treatment	df	F-value	p	Mean (± se)	Dunnett's test
Loudetiopsis	Nitrogen	62	1.41	0.24	6.67 (±0.79) ↑	0.08
	Water	62	5.07	0.03	7.33 (±0.90) ↑	0.02
	Nitrogen x Water	62	3.85	0.05	6.10 (±1.89) ↑	0.04
Tristachya	Nitrogen	42	0.77	0.38	1.42 (±0.26) ↓	0.85
	Water	42	<0.00	0.97	1.59 (±0.24) ↓	0.54
	Nitrogen x Water	42	0.81	0.37	1.59 (±0.34) ↓	0.86

Table 3-4. Total number of spikelets by treatment and species. Statistics are from a generalized model with negative binomial response variable distribution with nitrogen, water, and water x nitrogen as fixed effects. Means are mean number of total spikelets for plots with nitrogen or water , and plots with water and nitrogen. Model fit AIC = 644.87, χ^2 = 76.56 /df = 1.23, n = 66, (*Loudetiopsis*); AIC = 376.61, χ^2 = 61.39/df = 1.57, n =4 6 (*Tristachya*). Arrows indicate direction of effect relative to controls.

Species	Treatment	df	F-value	p	Mean (± se)	Dunnett's test
Loudetiopsis	Nitrogen	62	1.71	0.20	53.03 (± 8.39) ↑	0.03
	Water	62	4.77	0.03	58.71 (± 9.84) ↑	0.09
	Nitrogen x Water	62	1.16	0.29	60.32 (±12.89) ↑	0.01
Tristachya	Nitrogen	39	0.01	0.92	26.89 (± 4.73) ↓	0.97
	Water	39	0.91	0.35	29.96 (± 4.87) ↓	0.60
	Nitrogen x Water	39	2.14	0.15	35.58 (± 8.31) ↑	0.87

Table 3-5. Number of spikelets per flowering tiller by treatment and species. Statistics are from a general linear model with nitrogen, water, and nitrogen x water as fixed effects. Means are the average number of spikelets per tiller of flowering, for all plots with nitrogen or water, and plots with water and nitrogen. Model fit: $R^2 = 0.07$, p-value = 0.24, n = 66 (*Loudetiopsis*); $R^2 = 0.05$, p-value = 0.51, n = 43 (*Tristachya*). Arrows indicate direction of difference related to controls.

Species	Treatment	df	F-value	P	Mean (± se)	Dunnett's test
Loudetiopsis	Nitrogen	1	3.32	0.07	7.01 (± 1.08) ↑	0.77
	Water	1	0.11	0.74	6.11 (± 1.08) ↓	0.65
	Nitrogen x Water	1	0.88	0.35	7.10 (± 1.10) ↑	0.65
Tristachya	Nitrogen	1	0.42	0.52	13.54 (± 1.16) ↑	0.56
	Water	1	1.75	0.19	14.66 (± 1.15) ↑	0.52
	Nitrogen x Water	1	0.19	0.67	16.31 (± 1.22) ↑	0.15

Table 3-6. Difference in diameter between beginning and end of experiment by species and treatment. Statistics are from a general linear model with nitrogen, water, nitrogen x water (treatments), original diameter, and average PAR (covariates) as fixed effects. Interactions between treatments and covariates were included in the model when significant. Means are the centimeters of diameter difference for treatments from model with covariates. Model fit for treatment effects: $R^2 = 0.23$, p = 0.003, n = 77, (*Loudetiopsis*); $R^2 = 0.13$, p = 0.07, n = 77 (*Tristachya*).

Species	Treatment	df	F-value	p	Dunnett's test	Mean (± se)
Loudetiopsis	Nitrogen	1	1.62	0.20	0.88	1.25 (±1.12) ↓
	Water	1	0.13	0.72	0.90	1.42 (±1.12) ↓
	Nitrogen x Water	1	1.30	0.26	0.22	1.11 (±1.18) ↓
	Original Diameter	1	8.08	<0.01	↓	
	Original Diameter x Nitrogen	1	3.74	0.06	↓	
	Average PAR	1	5.70	0.02	↑	
Tristachya	Nitrogen	1	3.76	0.06	0.13	3.22 (±1.12) ↑
	Water	1	0.06	0.80	0.96	2.77 (±1.12) ↑
	Nitrogen x Water	1	0.68	0.41	0.53	2.96 (±1.18)↑
	Original Diameter	1	3.73	0.06	↓	
	Average PAR	1	4.08	0.05	↑	

Table 3-7. Root:shoot ratios by species and treatment. Statistics are from a general linear model. Means are for root:shoot ratio for all plants with nitrogen or water, and plants with just nitrogen and water. Model fit: $R^2 = 0.09$, $p = 0.07$, $n = 80$ (*Loudetiopsis*); $R^2 = 0.003$, $p = 0.98$, $n = 79$ (*Tristachya*).

Species	Treatment	df	F-value	p	Mean (± se)	Dunnett's test
	Nitrogen	1	3.06	0.08	0.93 (± 1.09) ↓	0.39
Loudetiopsis	Water	1	4.16	0.04	0.91 (± 1.09) ↓	0.52
	Nitrogen x Water	1	0.01	0.91	0.82 (± 1.12) ↓	0.02
	Nitrogen	1	0.18	0.67	1.20 (± 1.08) ↓	0.99
Tristachya	Water	1	0.02	0.88	1.17 (± 1.11) ↑	0.98
	Nitrogen x Water	1	<0.01	0.98	1.19 (± 1.11) ↑	0.99

Table 3-8. Live leaf (LL), and dead aboveground biomass (DAB), and total aboveground biomass (TAB, g dry weight), each divided by diameter by treatment and species. Model is a general linear model. Fit statistics are embedded in the table. *Loudetiopsis*: n = 79; *Tristachya*: n = 79.

			DAB			LL			TAB (LL + DAB)		
Species	Treatment	df	F-value	p	Dunnett's test	F-value	p	Dunnett's test	F-value	p	Dunnett's test
Loudetiopsis	Nitrogen	1	0.08	0.78	1.00 =	2.12	0.15	0.90↑	1.92	0.17	0.81 ↑
	Water	1	6.92	0.01	0.23 ↑	1.72	0.19	0. 94↑	5.12	0.03	0.40 ↑
	Nitrogen x Water	1	0.09	0.76	0.11 ↑	0.42	0.52	0.13 ↑	0.13	0.72	0.03 ↑
		78	2.36	0.08	R^2 = 0.09	1.44	0.24	0.05	2.42	0.09	R^2 = 0.09
Tristachya	Nitrogen	1	0.48	0.49	0.42↓	1.13	0.29	0.47↓	0.98	0.33	0.41↓
	Water	1	0.91	0.33	0.31↓	0.15	0.70	0.79↓	0.45	0.50	0.56↓
	Nitrogen x Water	1	1.33	0.25	0.49↓	0.35	0.50	0.61↓	0.78	0.38	0.50↓
	Model Fit	78	0.93	0.43	R^2 = 0.06	0.59	0.63	R^2 = 0.02	0.74	0.50	R^2 = 0.05

Table 3-9. Number of live leaves per area (cm^2) by treatment and species. Statistics derive from an analysis of covariance with nitrogen and water and their interaction as fixed effects and diameter as a continuous covariate. Model fit: $R^2 = 0.14$, p = 0.02, n = 80 (*Loudetiopsis*); $R^2 = 0.06$, p =0.23, n = 80 (*Tristachya*).

Species	Treatment	df	F-value	p	Mean (± se)	Dunnett's test
Loudetiopsis	Nitrogen	1	0.48	0.49	0.27 (± 0.88) ↑	0.74
	Water	1	4.80	0.03	0.31 (± 1.13) ↑	0.98
	Nitrogen x Water	1	0.32	0.95	0.33 (±1.19) ↑	0.50
	Diameter	1	6.99	0.01	↓	
Tristachya	Nitrogen	1	0.02	0.88	0.10 (± 1.11) =	0.99
	Water	1	0.23	0.63	0.10 (± 0.89) ↓	0.99
	Nitrogen x Water	1	0.03	0.87	0.10 (± 1.16) ↓	0.99
	Diameter	1	24.93	<0.0001	↓	

Table 3-10. Foliar nitrogen (g/Kg) by treatment and species. Model is an analysis of covariance with live leaf biomass as a covariate in the model, and treatments as fixed effects. *Loudetiopsis*: n = 80; *Tristachya*: n=79. Model fit statistics are embedded in the table.

Species	Treatment/Factor	F-value	p	Means (± se)	Dunnett's test
Loudetiopsis	Nitrogen	1.62	0.21	6.87 (± 0.10) ↑	0.99
	Water	0.39	0.53	6.82 (± 0.10) ↑	0.97
	Water x Nitrogen	1.35	0.25	7.00 (±0.15) ↑	0.41
	Live Leaves	4.77	0.03	↓	↓
	Model Fit	1.91	0.12		$R^2 = 0.09$
Tristachya	Nitrogen	0.40	0.53	7.74 (± 0.14) ↑	0.77
	Water	0.69	0.41	7.59 (± 0.14) ↓	0.99
	Water x Nitrogen	0.24	0.63	7.61 (±0.20) ↓	0.99
	Live Leaves	0.07	0.79	↓	
	Model Fit	0.36	0.84		$R^2 = 0.02$

Table 3-11. Foliar phosphorus (g/Kg) by treatment and species. Model is an analysis of covariance with live leaf biomass as a covariate in the model, and treatments as fixed effects. *Loudetiopsis*: n=80; *Tristachya*: n=79.

Species	Treatment	F-value	p	Means (± se)	Dunnett's test
	Nitrogen	1.78	0.19	0.66 (± 0.018) ↓	0.09
	Water	1.17	0.28	0.67 (± 0.018) ↓	0.13
Loudetiopsis	Water x Nitrogen	3.01	0.09	0.67 (± 0.025) ↓	0.22
	Live Leaves	1.96	0.17	↑	
	Model Fit	1.71	0.16		$R^2 = 0.08$
	Nitrogen	2.93	0.09	0.87 (± 0.020) ↑	0.40
	Water	3.12	0.08	0.87 (± 0.020) ↑	0.39
Tristachya	Water x Nitrogen	0.02	0.88	0.90 (± 0.028) ↑	0.04
	Live Leaves	0.21	0.65	↓	
	Model Fit	1.70	0.16		$R^2 = 0.08$

Table 3-12. N:P ratios by treatment and species. Model is an analysis of covariance with live leaf biomass as a covariate in the model, and treatments as fixed effects. *Loudetiopsis*: n = 80; *Tristachya*: n = 79.

Species	Treatment	F-value	p	Means (± se)	Dunnett's test
	Nitrogen	3.74	0.06	10.40 (± 1.03) ↑	0.09
	Water	1.70	0.20	10.28 (± 1.03) ↑	0.23
Loudetiopsis	Water x Nitrogen	1.18	0.28	10.44 (± 1.03) ↑	0.07
	Live Leaves	7.34	0.01	↓	
	Model Fit	2.91	0.03		$R^2 = 0.13$
	Nitrogen	1.57	0.21	8.89 (± 1.03) ↓	0.71
	Water	5.87	0.02	8.70 (± 1.03) ↓	0.23
Tristachya	Water x Nitrogen	<0.01	0.99	8.51 (± 1.04) ↓	0.03
	Live Leaves	0.02	0.90	↑	
	Model Fit	1.90	0.12		$R^2 = 0.09$

Table 3-13. Response of soil phosphorus concentration (g/Kg of dry soil) to treatments and species. Model is a generalized linear model with gamma distribution. Means presented are for comparisons of watered and un-watered, plots with and without nitrogen, plots with nitrogen and water, species effects, and significant interactions between species and treatment. Arrows indicate difference relative to controls. Model fit: AIC = values: -251.26, χ^2 = 11.94 /df = 0.16, n=80.

Treatment	df	F-value	p	Means (+ se)	Dunnett's test
Nitrogen	1	4.16	0.04	0.14 (+ <0.01) ↑	0.12
Water	1	0.04	0.84	0.13 (+ <0.01) ↑	0.82
Nitrogen x Water	1	0.64	0.43	0.14 (+ 0.01) ↑	0.27
Species	1	2.68	0.06	*Loudetiopsis*: 0.12 (+ < 0.01) *Tristachya*: 0.14 (+ < 0.01)	- -
Water * Species	1	7.37	<0.01	*Loudetiopsis*: 0.11 (+ < 0.01) ↓ *Tristachya*: 0.16 (+ 0.01) ↑	- -

Table 3-14. Response of soil phosphorus concentration (g/Kg of dry soil) to treatments by species. Model is a generalized linear model with gamma response variable distribution. Means presented are for comparisons of watered and un-watered, plots with and without nitrogen, and plots with nitrogen and water. Arrows indicate difference relative to controls. Model fit: AIC = -131.42, χ^2 = 5.75 /df = 0.16, n = 40 (*Loudetiopsis*); AIC = -114.17, χ^2 = 6.18 χ^2/df =0.17, n=40 (*Tristachya*).

Species	Treatment	df	F-value	p	Mean (+ se)	Dunnett's test
Loudetiopsis	Nitrogen	36	2.97	0.11	0.13 (+0.011) ↑	0.09
	Water	36	1.87	0.07	0.11 (+ 0.009) ↓	0.47
	Nitrogen x Water	36	0.63	0.43	0.11 (+ 0.013) ↓	0.89
Tristachya	Nitrogen	36	1.56	0.22	0.15 (+ 0.013) ↑	0.27
	Water	36	3.99	0.05	0.16 (+ 0.014) ↑	0.11
	Nitrogen x Water	36	0.12	0.73	0.17 (+ 0.020) ↑	0.03

Table 3-15. Analysis of pH, aluminum, potassium, and calcium response to treatments. Statistics are for a general linear model with nitrogen and water as fixed effects (n = 80 for all variables). Means presented are for comparisons of watered and un-watered, nitrogen fertilized and unfertilized plots, and nitrogen x water plots. Arrows indicate direction of difference relative to controls. Model fit statistics are embedded in the table.

	pH (in H_2O)				Aluminum (g/Kg dry soil)			
Treatment	df	F -value	p	Means (± se)	Dunnett's test	F -value	p	Means (± se)	Dunnett's test
Nitrogen	1	0.38	0.54	4.93 (± 0.04)	0.85 ↓	1.60	0.21	0.064 (± 0.002)	0.28 ↑
Water	1	0.05	0.83	4.94 (± 0.04)	0.96 ↓	1.09	0.30	0.061 (± 0.002)	0.99 ↓
Nitrogen x Water	1	0.10	0.75	4.93 (± 0.05)	0.88 ↓	0.89	0.35	0.062 (± 0.003)	0.99 ↑
Species	1	2.24	0.14	L: 4.90 (± 0.04) T: 4.80 (± 0.04)		0.74	0.39	L: 0.064 (± 0.002) T: 0.061 (± 0.002)	
Model Fit	79	0.69	0.60		$R^2 = 0.06$	1.08	0.37		$R^2 = 0.06$

	Potassium (g/Kg dry soil)			Calcium (g/Kg dry soil)			
F-value	p	Means (± se)	Dunnett's test	F-value	p	Means (± se)	Dunnett's test
3.00	0.09	8.25 (± 1.01)	0.23 ↓	3.15	0.08	0.012 (±1.11)	0.23 ↑
0.08	0.77	8.25 (± 1.01)	0.85 ↓	0.09	0.76	0.011 (± 1.11)	0.86 ↑
0.42	0.52	8.25 (± 1.01)	0.35 ↓	0.36	0.55	0.012 (± 1.16)	0.32 ↑
2.49	0.12	L: 8.33 (± 1.01) T: 8.25 (± 1.01)		0.01	0.93	L: 0.011 (± 1.11) T: 0.010 (± 1.11)	
1.29	0.28		$R^2 = 0.07$	0.90	0.47		$R^2 = 0.05$

Table 3-16. Results of resin available ammonium (μg/ml in 28 days) response to treatments and species. Statistics are for a generalized linear model with a gamma response variable distribution with nitrogen, water, their interaction, and species as fixed effects. Means presented are for species and treatments. Model fit: AIC = 164.53, $\chi 2 = 46.24$ /df = 0.65, n = 76.

Treatment	df	F-value	p	Mean (± se)	Dunnett's test
Nitrogen	1	<0.001	0.98	1.10 (± 0.13) ↑	0.48
Water	1	4.72	0.03	1.31 (± 0.15) ↑	0.02
Nitrogen x Water	1	1.16	0.28	1.20 (± 0.20) ↑	0.14
Species	1	0.18	0.67	*Loudetiopsis*: 1.14 (± 0.15) *Tristachya*: 1.06 (± 0.12)	

Table 3-17. Results of resin available nitrate (μg/ml in 28 days) response to treatments and species. Statistics are for a general linear model with nitrogen, water, their interaction, and species as fixed effects. Means presented are for treatments and species. Model fit: $R^2 = 0.06$, F-value: 1.13, p = 0.35, n =76.

Treatment	df	F-value	p	Means (± se)	Dunnett's test
Nitrogen	1	<0.001	0.99	0.98 (± 1.13) ↑	0.86
Water	1	3.70	0.06	1.14 (± 1.14) ↑	0.12
Nitrogen x Water	1	0.83	0.37	0.90 (± 0.83) ↑	0.30
Species	1	0.01	0.93	*Loudetiopsis*: 0.81 (± 0.82) *Tristachya*: 0.83 (± 1.13)	

Table 3-18. Results of ammonium mineralization rate (μg N/g over 7 days) response to treatments and species. Statistics are for a general linear model with nitrogen, water, their interaction, and species as fixed effects. Means presented are for treatments and species. Model Fit: $R^2 = 0.02$, F-value: 0.36, p = 0.84, n = 99.

Treatment	df	F-value	p	Means (± se)	Dunnett's test
Nitrogen	1	0.06	0.81	-0.72 (± 0.41) ↑	0.63
Water	1	<0.01	0.96	-0.78 (± 0.41) ↑	0.72
Nitrogen x Water	1	1.36	0.25	-1.05 (± 0.58) ↑	0.99
Species	1	0.03	0.86	*Loudetiopsis*: -0.84 (± 0.41) *Tristachya*: -0.74 (± 0.41)	

Table 3-19. Results of nitrate mineralization rate (μg N/ml in 7 days) response to treatments and species. Statistics are for a general linear model with nitrogen, water, their interaction, and species as fixed effects. Means presented are for treatments and species. Model Fit: $R^2 = 0.001$, F-value: 0.03, p = 0.99, n = 99.

Treatment	df	F-value	p	Means (± se)	Dunnett's test
Nitrogen	1	<0.01	0.99	0.05 (± 0.04) ↓	0.99
Water	1	0.03	0.87	0.06 (± 0.04) =	1.00
Nitrogen x Water	1	0.03	0.86	0.06 (± 0.06) ↑	0.99
Species	1	0.07	0.80	*Loudetiopsis*: 0.06 (± 0.04) *Tristachya*: 0.04 (± 0.04)	

Table 3-20. Results of soil ammonium (μg N/g) response to treatments and species. Statistics are for a general linear model with nitrogen, water, their interaction, and species as fixed effects. Means presented are for treatments and species. Model Fit: $R^2 = 0.02$, F-value: 0.38, p = 0.82, n = 98.

Treatment	df	F-value	p	Means (± se)	Dunnett's test
Nitrogen	1	0.21	0.65	2.15 (± 1.14) ↓	0.74
Water	1	0.72	0.40	2.43 (± 1.14) ↑	0.99
Nitrogen x Water	1	0.12	0.73	2.31 (± 1.20) ↓	0.99
Species	1	0.53	0.47	*Loudetiopsis*: 2.17 (± 1.14) *Tristachya*: 2.49 (± 1.14)	

Table 3-21. Results of soil nitrate (μg N/g) response to treatments and species. Statistics are for a general linear model with nitrogen, water, their interaction, and species as fixed effects. Means presented are for treatment and species. Model Fit: $R^2 = 0.05$, F-value: 1.08, p = 0.37, n = 98.

Treatment	df	F-value	p	Means (± se)	Dunnett's test
Nitrogen	1	0.52	0.47	0.08 (± 1.23) ↑	0.77
Water	1	3.53	0.06	0.05 (± 1.22) ↓	0.59
Nitrogen x Water	1	0.16	0.69	0.06 (± 1.33) ↓	0.75
Species	1	0.09	0.76	*Loudetiopsis*: 0.07 (± 1.22) *Tristachya*: 0.07 (± 1.23)	

Table 4-1. Table of significant results. The significance level is represented by * $0.05 < p \leq 0.10$ and ** $p \leq 0.05$.

Treatment	*Loudetiopsis*		*Tristachya*		Soil
	Reproductive	Vegetative	Reproductive	Vegetative	
Nitrogen	↑ * Spikelets/ flowering tiller	↑ * Foliar N:P ↓ * Root:shoot ↓ * Diameter difference	—	↑ ** Diameter difference ↑ * Foliar P	↑ * Ca ↓ * K ↑ * P
Water	↑ ** Flowering tillers ↑ ** Total spikelets	↓ ** Root:shoot ↑ ** Total aboveground biomass ↑ ** Dead aboveground biomass ↓** Senescence	↑ ** Flowering likelihood	↓** Foliar N:P ↑ * Foliar P	↑ **resin NH_4^+ ↑ * resin NO_3^- ↓ * soil NO_3^- ↑* P (*L.*) ↓** P (*T.*)
Water x Nitrogen	↑ ** Flowering likelihood ↑ ** Flowering tillers	↓ * Foliar P	—	—	—

APPENDIX A
TABLE OF UNIVARIATE STATISTICS

Treatment	*Loudetiopsis*				*Tristachya*			
	Min. Value	Mean	Median	Max. Value	Min. Value	Mean	Median	Max. Value
Live Leaf Biomass (g)								
Control	5.50	16.8	16.8	29.2	38.2	118.1	96.7	298.6
Nitrogen	1.9	20.4	18.7	50.1	25.9	94.1	84.0	187.0
Water	7.9	21.0	20.3	49.6	0.0	91.1	87.8	214.7
Water x Nitrogen	6.9	22.0	20.2	39.2	22.1	92.2	72.3	267.2
Dead Leaf Biomass (g)								
Control	11.7	25.4	22.5	51.1	63.5	291.4	238.7	749.2
Nitrogen	2.92	28.4	28.1	71.2	43.2	229.6	201.8	648.1
Water	13.0	43.2	31.7	145.7	60.3	189.9	140.2	716.8
Water x Nitrogen	13.4	36.1	28.3	82.8	38.0	226.2	142.9	810.8
Reproductive Tiller Biomass (g)								
Control	0.0	4.0	2.4	15.9	0.0	7.0	0.0	63.5
Nitrogen	0.0	6.1	3.0	25.4	0.0	6.9	0.0	35.8
Water	0.0	5.4	2.3	34.8	0.0	13.9	12.0	48.3
Water x Nitrogen	0.0	7.1	3.7	21.7	0.0	11.1	5.9	81.7
Root Biomass (g)								
Control	20.5	59.7	54.4	154.7	160.4	441.8	336.1	778.8
Nitrogen	11.31	59.6	55.4	134.0	71.5	418.7	336.4	1342.0
Water	14.7	71.8	66.6	229.1	56.5	362.6	325.7	814.9
Water x Nitrogen	22.0	53.8	50.3	132.9	102.0	363.3	274.4	1020.2
Total Aboveground Biomass (g)								
Control	22.1	46.2	43.0	93.7	116.9	416.5	374.9	1047.7
Nitrogen	11.5	54.9	56.3	111.8	69.1	330.6	280.5	835.1
Water	23.6	69.6	56.7	209.2	97.6	294.8	247.8	979.8
Water x Nitrogen	27.2	65.2	56.3	143.7	60.1	329.5	223.4	1090.1
Dead Tillers								
Control	0	14.9	14.0	36	6	17.4	15.0	61
Nitrogen	2	14.6	8.0	72	3	14.2	12.0	41
Water	4	22.2	12.5	88	3	15.1	13.0	65
Water x Nitrogen	1	17.9	14.5	45	1	15.1	9.0	45
Live Flowering and Non-flowering Tillers								
Control	24	63.1	67.5	109	17	47.5	38.0	112
Nitrogen	21	65.4	62.0	261	16	46.0	42.0	84
Water	31	81.5	75.0	149	12	46.7	40.0	110
Water x Nitrogen	34	60.7	60.0	112	13	46.9	35.5	118

Treatment	*Loudetiopsis*				*Tristachya*			
	Min. Value	Mean	Median	Max. Value	Min. Value	Mean	Median	Max. Value
Average PAR								
Control	285	1532	1742	1998	338	1626	1719	2068
Nitrogen	193	1525	1749	2041	274	1438	1695	2943
Water	222	1518	1556	2012	271	1581	1767	1963
Water x Nitrogen	762	1546	1754	1968	391	1746	1890	2089
Number of Flowering Individuals (2009)								
Control	-	18	-	-	-	9	-	-
Nitrogen	-	16	-	-	-	9	-	-
Water	-	13	-	-	-	14	-	-
Water x Nitrogen	-	19	-	-	-	14	-	-
Number of Flowering Tillers per Flowering Individual								
Control	1	3.7	3.0	12	1	2.0	2.0	4
Nitrogen	1	6.5	5.0	16	1	1.2	1.0	2
Water	1	7.8	6.0	24	1	1.6	1.0	4
Water x Nitrogen	1	6.8	7.0	16	1	1.6	1.0	6
Total Number of Spikelets								
Control	3	26.7	15.5	121	2	28.7	24.0	97
Nitrogen	4	46.6	37.5	129	1	19.3	16.0	51
Water	2	57.2	39.0	281	5	25.2	18.0	79
Water x Nitrogen	4	60.3	34.0	183	8	35.6	25.5	184
Number of Spikelets per Flowering Tiller								
Control	3	6.6	6.8	11.8	2	12.8	12.0	24.3
Nitrogen	4	7.4	6.7	13.3	1	14.9	14.0	25.5
Water	2	5.9	5.3	11.7	5	15.0	17.0	23
Water x Nitrogen	3.5	7.8	8.1	17.0	8	18.1	14.0	34
Original Diameter (cm)								
Control	5.2	9.1	8.9	14.8	14.6	25.0	23.6	42.8
Nitrogen	5.9	10.5	9.6	20.3	12.5	24.4	23.5	38.6
Water	6.2	11.0	10.1	17.4	11.1	22.7	20.4	46.7
Water x Nitrogen	4.7	9.4	9.1	17.8	10.8	21.7	21.9	36.2
Final Diameter (cm)								
Control	6.1	10.9	10.9	17.6	18.2	28.1	27.6	45.9
Nitrogen	4.8	11.9	10.5	20.8	15.2	28.1	26.4	43.6
Water	6.4	13.0	12.9	20.7	13.7	26.1	23.2	50.5
Water x Nitrogen	6.7	10.3	10.2	15.8	13.2	25.3	25.4	39.15.6

Treatment	*Loudetiopsis*				*Tristachya*			
	Min. Value	Mean	Median	Max. Value	Min. Value	Mean	Median	Max. Value
Root:shoot Ratio								
Control	0.50	1.56	1.07	4.67	0.37	1.31	1.29	2.32
Nitrogen	0.46	1.24	0.98	4.79	0.24	1.35	1.38	2.55
Water	0.47	1.16	1.03	2.97	0.53	1.24	1.20	2.49
Water x Nitrogen	0.42	0.90	0.87	2.31	0.51	1.27	1.21	1.92
Foliar Nitrogen (g/Kg)								
Control	5.60	6.78	6.86	7.70	6.30	7.64	7.63	8.82
Nitrogen	5.60	6.73	6.65	8.26	5.60	7.88	8.05	10.01
Water	5.46	6.63	6.65	7.56	6.02	7.58	7.42	10.01
Water x Nitrogen	5.60	6.97	7.00	8.40	6.16	7.61	7.39	10.36
Foliar Phosphorus (g/Kg)								
Control	0.50	0.73	0.72	1.02	0.48	0.79	0.79	1.07
Nitrogen	0.40	0.65	0.67	0.88	0.64	0.85	0.88	1.00
Water	0.49	0.66	0.64	0.87	0.70	0.85	0.84	1.15
Water x Nitrogen	0.56	0.67	0.69	0.98	0.68	0.90	0.89	1.14
Foliar N:P								
Control	6.5	9.5	9.3	12.4	7.4	9.9	9.4	14.3
Nitrogen	8.2	10.5	10.0	15.6	5.6	9.4	9.4	13.3
Water	6.6	10.2	10.4	13.6	7.4	9.0	8.7	12.2
Water x Nitrogen	6.6	10.5	10.3	13.7	6.9	8.6	8.1	11.9
Soil Phosphorus (g/Kg)								
Control	0.08	0.11	0.09	0.18	0.06	0.12	0.11	0.19
Nitrogen	0.08	0.15	0.14	0.32	0.08	0.14	0.12	0.29
Water	0.05	0.10	0.10	0.16	0.08	0.15	0.16	0.26
Water x Nitrogen	0.06	0.11	0.11	0.24	0.08	0.17	0.17	0.34
pH (in water)								
Control	4.61	4.95	4.97	5.32	4.50	4.92	4.90	5.29
Nitrogen	4.56	4.83	4.83	4.99	4.75	4.97	4.97	5.24
Water	4.42	4.98	5.04	5.36	4.49	4.98	5.01	5.58
Water x Nitrogen	4.70	4.93	4.96	5.11	4.49	4.94	5.10	5.31
Calcium (g/Kg)								
Control	0.0045	0.0099	0.0072	0.0280	0.0027	0.0106	0.0090	0.0208
Nitrogen	0.0063	0.0203	0.0154	0.0814	0.0036	0.0108	0.0090	0.0208
Water	0.0054	0.0097	0.0099	0.0154	0.0036	0.0162	0.0099	0.0515
Water x Nitrogen	0.0045	0.0122	0.0104	0.0235	0.0045	0.0199	0.0099	0.0787

Treatment	*Loudetiopsis*				*Tristachya*			
	Min. Value	Mean	Median	Max. Value	Min. Value	Mean	Median	Max. Value
Potassium (g/Kg)								
Control	8.07	8.36	8.45	8.52	8.06	8.35	8.37	8.61
Nitrogen	7.51	8.19	8.23	8.50	7.63	8.26	8.36	8.53
Water	8.16	8.41	8.41	8.65	7.75	8.21	8.18	8.49
Water x Nitrogen	7.82	8.36	8.40	8.59	7.43	8.13	8.11	8.51
Aluminum (g/Kg)								
Control	0.043	0.063	0.063	0.081	0.041	0.059	0.063	0.0678
Nitrogen	0.054	0.068	0.068	0.081	0.047	0.065	0.063	0.101
Water	0.045	0.063	0.060	0.081	0.032	0.059	0.065	0.072
Water x Nitrogen	0.043	0.063	0.061	0.081	0.041	0.060	0.059	0.086
Resin Available Nitrate (μg N/28 days)								
Control	0.125	0.351	0.255	0.961	0.010	0.164	0.141	0.344
Nitrogen	0.016	0.441	0.293	1.033	0.007	0.240	0.213	0.950
Water	0.006	0.336	0.161	0.828	0.008	0.217	0.161	0.503
Water x Nitrogen	0.112	0.493	0.355	1.338	0.027	0.127	0.098	0.232
Resin Available Ammonium (μg N/28 days)								
Control	0.178	1.03	0.554	2.732	0.239	0.707	0.651	1.447
Nitrogen	0.293	0.786	0.538	1.911	0.311	1.117	0.703	3.619
Water	0.136	1.222	1.171	2.493	0.364	1.680	1.530	4.705
Water x Nitrogen	0.644	1.685	1.401	4.068	0.128	0.939	0.639	2.032
Soil Nitrate (μg N/g)								
Control	0.008	0.129	0.114	0.302	<0.001	0.080	0.086	0.186
Nitrogen	<0.001	0.165	0.105	0.823	0.058	0.247	0.105	1.211
Water	<0.001	0.067	0.078	0.163	0.014	0.145	0.097	0.502
Water x Nitrogen	0.019	0.138	0.111	0.329	<0.001	0.069	0.091	0.119
Soil Ammonium (μg N/g)								
Control	1.474	2.433	2.236	4.107	1.380	2.832	2.572	4.949
Nitrogen	<0.001	2.729	2.181	4.993	1.286	3.139	2.918	6.335
Water	1.191	2.622	2.186	5.442	1.059	3.133	2.541	6.633
Water x Nitrogen	1.568	2.961	2.923	4.905	1.131	2.068	1.984	3.209
Nitrate Mineralization Rate (μg N/7 days)								
Control	-0.141	0.045	-0.033	0.579	-0.186	0.057	-0.050	1.078
Nitrogen	-0.485	0.121	0.089	0.989	-1.211	-0.038	0.019	0.551
Water	-0.163	0.019	-0.007	0.407	-0.418	0.088	0.028	0.961
Water x Nitrogen	-0.330	0.059	-0.039	1.139	-0.114	0.064	-0.025	0.629

Treatment	*Loudetiopsis*				*Tristachya*			
	Min. Value	Mean	Median	Max. Value	Min. Value	Mean	Median	Max. Value
Ammonium Mineralization Rate (μg N/7 days)								
Control	-3.827	-0.606	-1.571	6.631	-4.315	-1.831	-1.943	2.214
Nitrogen	-3.395	0.434	0.418	6.753	-4.320	-1.225	-1.934	9.751
Water	-4.652	-1.473	-1.300	1.976	-4.395	0.604	-0.266	14.518
Water x Nitrogen	-4.791	-1.581	-1.623	2.602	-3.034	-0.552	-0.917	5.052

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BIOGRAPHICAL SKETCH

Stella Copeland grew up in the Bay Area for the first few years of her life, but the formative ones were spent in the mountains and streets of small town Ashland, Oregon. A senior year in a public school program called Wilderness Charter School gave her the freedom to explore botany and organic gardening. Taking a leap east, she ended up at Colorado College for an amazing four years of a liberal arts education with lots of field science and a dash of everything from ethnography to sociology of immigration. To make a buck, and follow her muse, she quit her cushy job as an usher at the Oregon Shakespeare Festival, and began working for the Bureau of Land Management, in Medford OR during summers off from CC. A season of rare plant surveys in one of the botanical hotspots of the US, and she was hooked on plant ecology. A study abroad in Ecuador clinched the matter, especially after a month in the cloud forest collecting rare orchids. Plants now on the brain, she worked for the Colorado Natural Heritage Program in the imposing, wild Sangre de Cristo mountain range, then headed south for the winter to Peru for another orchid oriented position. Northward, she spent a year as head of a small botany and forestry monitoring crew for the Nature Conservancy, then to Argentina to be a tourist and teach English for three months. After a brief stint as a organic farm intern and a rare plant contractor in Ashland, she headed off to Florida where she has spent the last two years chasing academic dreams, learning Portuguese, delving into ecosystem ecology, and generally coming to the realization that she may not know much, but the trick is to make peace with ignorance. In the spirit of learning, and going forward in the field of conservation ecology, she is now headed to the University of California, Davis, in search of a PhD, and the secrets of declining rare plants.