FINAL COMPLETION REPORT

Title: Impacts of Atmospheric Nitrogen Deposition and Climate Change on Desert Ecosystems
PMIS#: 83909, Big Bend National Park, National Park Service.

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Duration of Project: 2003-2006

Project Summary

A major concern for management of the National Parks is the potential change in the magnitude and timing of atmospheric chemical inputs and precipitation as a consequence of the accumulation of greenhouse gasses and their effect on global climate. Changes in atmospheric nitrogen deposition and precipitation frequency and amounts are likely to have significant effects on biodiversity, ecosystem productivity, nutrient dynamics, and groundwater and stream hydrology. Knowledge of how arid ecosystems are likely to respond to atmospheric nitrogen input and potential climate changes are needed to inform management policies at Big Bend National Park.

Our research at Big Bend National Park seeks to understand: 1) the impacts of nitrogen deposition on microbial and soil nitrogen dynamics and plant productivity within the sotol-grasslands and high elevation oak-pine forests within the Pine Canyon Watershed, and 2) the impacts of changes in precipitation timing and amounts on plant growth, soil nutrient and microbial dynamics in the sotol-grassland. This research effort is part of a larger 10 year effort at Big Bend National Park to examine impacts of anthropogenic impacts and climate change in Referenced Watershed sites that have been established by scientists within NPS and the USGS in five National Parks. The climate change research at Big Bend National Park is also part of a network of research sites (PrecipNet) that explore climate change impacts across the western United States.

The most widely accepted general circulation models (GCMs) have predicted a rise in the mean temperature of the Earth's surface from 1.4°C to 5.8°C during this century, which could trigger a subsequent increase in mean global precipitation of up to 7% (Houghton et al. 2001). In the southwestern United States and in particular in the Big Bend region, both the Hadley Climate Model 3 (HadCM3) and the Canadian Global Climate Model (CGCM) predicts a 3°C increase in air temperature by 2100, and a 25 % increase in precipitation. However, the two models differ as to when this increase in precipitation will occur during the year. The HadCM2 predicts an increase in summer precipitation and a decline in winter precipitation, whereas the CGCM suggests an increase in winter precipitation and a decline in summer precipitation. To capture the uncertainty in the GCMs predictions as to the season of precipitation increase, both summer and winter increases in precipitation were included in our experimental design.

A concern of land managers continues to be the impacts of nitrogen deposition on plant dynamics and diversity, soil nutrient dynamics, and microbial activity. Expanding fossil fuel use continues to increase atmospheric deposition across much of North America. Approximately 140TgN/yr is added to the soil around the world from anthropogenic sources. This amount is about equal to the estimated rate of natural N-fixation by bacteria (Johnson et al. 1991, Vitousek et al. 1997, and Friedland and Miller 1999). If nitrogen deposition continues to occur at rates that exceed ecosystem use the deposition results in a variety of effects that are collectively describes as Nitrogen Saturation (Aber et al. 1996; Magill et al. 1996). The effects of nitrogen saturation can range from increased incidence of invasive plants, to the leaching of cations into streams and ground water (Aber et al. 1998).

Although the amount of nitrogen deposition in arid ecosystems across the southwestern United States is typically less that that found for grasslands and forested systems in other parts of the country, N saturation can occur with even low amounts of N deposition depending upon the systems ability to either use or remove excess nitrogen (Fenn et al. 1998). However no threshold for nitrogen deposition levels leading to nitrogen saturation have been proposed for arid ecosystems. Moreover, the impacts of nitrogen deposition can potentially be more drastic in arid ecosystems than in more mesic ones given that arid ecosystems are typically water regulated and nitrogen limited and are more prone to disturbances which can quickly lead to desertification (Reynolds and Stafford-Smith 2002 and Vernon et al. 2006)

To address the impacts of precipitation changes and nitrogen deposition on arid ecosystems, this study was initiated in 2002 in the Sotol Grasslands and high elevation Oak-Pine Forest along the Pine Canyon Watershed in Big Bend National Park, in the Chihuahuan Desert. Plots to evaluate impacts of changes in precipitation amounts and frequency were established in the summer of 2002 in the Sotol Grasslands, while plots to evaluate impacts of nitrogen deposition were established in spring 2003 in the Sotol Grasslands and in the high elevation Oak-Pine Forest on Lost-Mine Peak. Constraints due to location prevented establishing water manipulation plots at the Lost Mine site. Funding from NPS for the project began in 2003.

To accommodate the uncertainty in timing of additional precipitation, water manipulations included the following treatments: 1) control – normal precipitation, 2) winter water, 3) summer water and 4) winter and summer water. Water additions occurred in February, at the end of the winter period, and during June, July and August for the summer watering events. During the first year of the study, we had designed to apply a 25% increase in the historic winter precipitation and historic monthly precipitation amounts for June, July and August. In discussions with other researchers involved in Precipnet, we changed the water amounts to reflect a 25% increase in previously obtained rainfall amounts to better track actual precipitation amounts. Thus, beginning in 2004 we applied a 25% increase in the amount of precipitation received from December through our water event in late February. If the period was dry, we applied 25% above the historic record. For the summer water events we added 25% above that received for the previous month. With this design we could more effectively track yearly changes in precipitation. Three 4x4 m plots were established in the Sotol Grassland for each water treatment. Each plot contained representative of the three dominant plants in the SotolGrasslands (*Dasylirion leiophyllu* – sotol, *Bouteloua curtipendula* –sideoats grama, and *Opuntia phaeacantha* –brown spined prickly pear). Additional thirty-six, 0.5 x1 m steel bases were placed around individual sotol, side oats grama and brown spined prickly pear to obtain whole system gas exchange measurements. The small plots were watered in the same manner as the larger community plots.

The amount of nitrogen added to the N-deposition plots was based on NADP data collected at Big Bend since 1980. For the previous twenty-two years the yearly average amount of N-deposition has been 3.99 kg/h/yr at the Panther Junction recording station. For the nitrogen deposition study thirty 5x5 m plots were established in the Sotol Grasslands and in the high elevation Oak-Pine forest on Lost Mine Peak in April 2003. Treatments for this study were: 1) control – background nitrogen deposition, 2) 2X – twice the yearly average N deposition, and 4) four times the annual nitrogen input. Nitrogen was added to the plots during June and July in 2003, 2004, 2005, and 2006. During each month half the yearly amount was added as NH_4NO_3 and $CaNO_3$ mixed with sand for broadcast on the plots. Each plot was surrounded by a 0.5 m buffer and to allow access to the plots. Thus there are ten replicates for each treatment at each location.

Project Objectives

The objectives of this study were: 1) to determine the impacts of increased precipitation, reflecting predicted future climate change scenarios, on the species composition and performance of plant and microbial communities within the sotol-grassland along Pine Canyon Watershed site at BBNP, and 2) assess the ecological effects of increased nitrogen deposition at the Sotol-grassland and high elevation oak-pine forest (Lost Mine site) along the Pine Canyon Watershed.

Experimental Design: *Changes in Precipitation Amount and Frequency*

Four water manipulation treatments were applied to community and single plant species plots (see summary information) that were established in the Sotol Grasslands along the Pine Canyon Watershed at Big Bend National Park. The water manipulation component of the project was modified after the first year based on discussion with other researchers at other arid system location to better capture yearly variation in seasonal precipitation amounts. Water applications subsequently tracked yearly differences in winter and summer precipitation amounts and timing. Sampling for microbial and soil nitrogen dynamics were performed one month after water application to capture long-term impacts of water additions. Plant productivity and growth were performed seasonally. ANPP was based on allometric relationships for the three dominant plants obtained from plants collected off plot. Plant photosynthesis and water use efficiencies were obtained during the ten days following the winter and summer water events and periodically during the year.

Parameters Measured: *Changes in Precipitation Amount and Frequency*

Community Plots

- Plant productivity – ANPP in response to winter, summer, and winter/summer precipitation increases
- Plant species composition –
- Photosynthesis rates and plant water use efficiency
- Bacterial and fungal functional diversity – ability to use a range of carbon compounds
- Microbial Biomass Carbon – the biomass of microorganisms in the soil
- Soil $NH_{4,}$ and NO_3 pool sizes
- Soil pH
- Soil organic matter levels
- Nitrogen mineralization potential – based on 40 and 80 day lab incubations

Soil and air temperatures
Continuous monitoring of soil moisture

Single Species Plots
Ecosystem fluxes of CO_2 and H_2O

Experimental Design: *Impacts of Nitrogen Deposition*

The impact of twice and four times the average annual nitrogen deposition was followed over three years in the Sotol Grassland and high elevation Oak-Pine Forest on Lost Mine Peak. Details are provided in the project summary session of this report. Soil sampling occurred in April of each year and in August, one month after last application of additional nitrogen. With this design the short and long-term impacts could be ascertained.

Parameters Measured: *Impacts of Nitrogen Deposition*
Bacterial and fungal functional diversity on carbon – ability to use a range of carbon compounds
Fungal functional diversity on nitrogen compounds – ability to use a range of organic and inorganic nitrogen compounds in response to nitrogen additions.
Microbial Biomass Carbon – the biomass of microorganisms in the soil
Soil NH_4, and NO_3 pool sizes
Soil pH
Soil organic matter levels
Soil and air temperature
Soil moisture at time of sampling
Continuous monitoring of soil moisture – ECHO probes

Accomplishments

Project Establishment

We were able to establish all of the experimental manipulations that were specified in the project description (2003 -2006).

Completed Graduate Student Theses or Dissertations Supported by this Project

1. Robertson, T. December 2006. Plant Productivity and Community Response to Three Years of Supplemental Precipitation in the Sotol Grasslands of Big Bend National Park, TX. Ph.D., Texas Tech University, 125 p.
2. Campbell, J. August 2006. Distribution of Oligotropic Bacteria Along an Elevational Gradient at Big Bend National Park, Ph.D. Dissertation, Texas Tech University, 185p.
3. Grizzle, H. W. May 2005. The Impacts of Nitrogen Deposition on Microbial Function and Edaphic Parameters in the Chihuahuan Desert. M.S. Thesis, Texas Tech University, 84p.
4. Resinger, J. S. December 2004. Understanding Nitrogen and Microbial Dynamics Associated with Two Degraded Grassland Systems in Big Bend National Park. M.S. Thesis, Texas Tech University, 84p.

On-Going Graduate Student Theses or Dissertations Supported by this Project

1. Lisa Patrick, Ph.D. Dissertation Title: "Effects of Increased Precipitation and Nitrogen Deposition on the Physiological Responses of Desert Plant Communities. In-Progress
2. Heath Grizzle, Ph.D. Dissertation Title: Long-term Impacts of Nitrogen Deposition on Soil Microbial and Nutrient Dynamics in Desert Ecosystems. In-Progress
3. Colin Bell. M.S. Impacts of Changing Precipitation Patterns on Soil Microbial Dynamics in a Desert Grassland.
4. Jeb Clark, M.S. Microbial Genetic Diversity Along a Desert Watershed. In-Progress

Published Manuscripts

1. Grizzle, H. and **J. C. Zak**. 2006. The Soil Nitrolog Procedure: Assessment of Fungal Functional Diversity on Nitrogen Compounds. Mycologia 98: 353-363.
2. Patrick, L. J. Cable, D. Potts, D. Ignace, G. Barron-Gafford, A. Griffith, H. Alpert, N. Van Gestel, T. Robertson, T. E. Huxman, **J. Zak**, M. E. Loik, and **D. Tissue** 2006. Effects of an Increase in Summer Precipitation on Leaf, Soil and Ecosystem Fluxes of CO_2 and H_2O in a Sotol Grassland in Big Bend National Park, Texas. Oecologia (Accepted)
3. **Zak, J. C.** 2005. Fungal Communities of Desert Ecosystems: Links to Climate Change. In: The Fungal Community: Its Organization and Role in the Ecosystem, 3rd edition. J. Dighton, J. F. White, and P. Oudemans. (eds), Taylor & Francis Group, Boca Roton.
4. Huxman, T. E., M.D. Smith, P. Fay, A.K. Knapp, M.R. Shaw, M.E. Loik, S.D. Smith, D.T. Tissue**, J.C. Zak**, J. F. Weltzin, W.T. Pockman, O. Sala, B. Haddad, J. Harte, G. W. Koch, S. Schwinning, E. Small, and D. G. Williams. 2004. Convergence across biomes to a common rain-use efficiency. Nature. 429: 651-654.
5. Sobek, E.A., **J. C. Zak**. 2003. The soil Fungilog procedure: method and analytical approaches towards understanding fungal functional diversity. Mycologia. 95:590-602.
6. Weltzin, J. F., **M. E. Loik**, S. Schwinning, D. G. Williams, P. Fay, B. Haddad, J. Harte, T. E. Huxman, A. K. Knapp, G. Lin, W. T. Pockman, M. R. Shaw, E. Small, M. D. Smith, S. D. Smith, **D. T. Tissue, and J. C. Zak**. 2003. Assessing the response of terrestrial ecosystems to potential changes in precipitation. Bioscience53: 941-952.

Presentations:

1. Bell, C., J. Zak, H. Grizzle, S. Cox, D. Tissue and J. Sirotnak. 2006. Microbial carbon and nitrogen mineralization dynamics in desert grassland soils of Big Bend National Park as influenced by global climate change. Ecological Society of America, Annual Meeting, Memphis, TN.
2. Bell, C.W. J. Zak, H. Grizzle, and D. Tissue. August 2006. Microbial carbon usage and nitrogen mineralization dynamics in desert grassland soils of Big Bend NP as influenced by global climate change. Annual meeting of the Ecological Society of America, Memphis, TN.
3. Campbell, J., J. Clark and J. Zak. August 2006. A comparison of microbial diversity as assessed by DGGE from fresh and archived soil samples. Annual meeting of the Ecological Society of America, Memphis, TN.
4. Ebbets, A., D. Barker, V. Ebbert, T. Rosenstiel, W. Adams, D. Tissue, and S. Smith. 2006. Potential changes in photosynthetic performance of two Mojave Desert shrubs in

response to elevated CO2. Ecological Society of America, Annual Meeting, Memphis, TN.

5. Grizzle, H. J. Zak and D. Tissue. August 2006. Impacts of nitrogen deposition on microbial activity in two desert ecosystems. Annual meeting of the Ecological Society of America, Memphis, TN.
6. Patrick, L. 2006 "Effects of increased precipitation on the carbon balance of North American desert plants" EPA Graduate Fellows Conference, Washington, DC, September 2006,
7. Patrick, L. and D. Tissue. 2006. How does summer precipitation affect daily CO2 and H_2O fluxes of North American desert plants? Ecological Society of America, Annual Meeting, Memphis, TN.
8. Tissue, D.T**.** 2006. Global climate change – impacts on plant physiological and ecosystem processes. ComBio Plenary Address, Brisbane, Australia.
9. Bell, C. W., H. W. Grizzle, and J. C. Zak. May 2005. Microbial responses to increased precipitation in the sotol grasslands of Big Bend National Park. Soil Ecology Society Tenth Biennial International Conference, Argonne National Laboratory.
10. Campbell, J.H., J. C. Zak, R. M. Jeter and R.E. Strauss. Effects of soil chemistry on distributions of oligotrophic bacteria along an elevational gradient. Soil Ecology Society Tenth Biennial International Conference, Argonne National Laboratory.
11. Clark, J. S., J. H. Campbell, and J. C. Zak. May 2005. Soil eubacterial diversity along a vegetation and elevation gradient in the Chihuahuan Desert, Big Bend National Park. Soil Ecology Society Tenth Biennial International Conference, Argonne National Laboratory.
12. Patrick, L., D. Tissue and J. Zak. August 2005. "Response of daytime net ecosystem carbon and water exchange to a large winter precipitation pulse in Big Bend National Park" - Ecological Society of America International Meeting, Montreal, Canada
13. Patrick, L. and D. Tissue. April 2005. "Photosynthetic responses of desert plants to changes in the timing and magnitude of precipitation at Big Bend National Park" Graduate Forum, Department of Biological Sciences, Texas Tech University.
14. Patrick, L. and D. Tissue. November 2005. "Effects of increased precipitation on the physiological responses of desert plant communities" - ARCS Scholar Reception, Lubbock, TX
15. J. Zak, H. Grizzle, C. Bell, J. Campbell, E. Sobek, and D. Tissue. May 2005. Microbial dynamics and processes in a desert landscape: Impacts of climate change, soil nutrients, and vegetation. Soil Ecology Society Tenth Biennial International Conference, Argonne National Laboratory.
16. Patrick, L., P. on, S. Lambrecht, J. zak, M. Loik, D. Tissue. 2004. Photosynthetic response of desert plants to a large, single precipitation event at Big Bend National Park. Ecological Society of America Annual Meeting, Portland, OR, August 2004.
17. Robertson, T., N. van Gestel, E. Walker, J. Zak, M. Loik and D. Tissue. 2004. Plant growth responses to simulated rainfall events for three perennial Chihuahuan Desert species of Big Bend National Park. Ecological Society of America Annual Meeting, Portland, OR, August 2004.
18. Campbell, J. H., E. A. Sobek, R. M. Jeter, and J. C. Zak. Distributions of oligotrophic bacteria along an elevational gradient at Big Bend National Park. Soil Ecology Society 9th Biannual Meeting, Palm Springs, CA May 2003.

19. Grizzle, H., D. Tissue, J. Sirotnak, and J. C. Zak. The impacts of simulated increased nitrogen deposition on functional diversity, biomass, and species richness of soil fungal assemblages in the Chihuahuan Desert at Big Bend National Park. Soil Ecology Society 9th Biannual Meeting, Palm Springs, CA May 2003.
20. Nagel, J. M., D. T. Tissue, and J. C. Zak. Physiological responses to changes in soil properties: potential impacts on desert plant communities in Big Bend National Park. Soil Ecology Society 9th Biannual Meeting, Palm Springs, CA May 2003.
21. Nagy, A., T. Robertson, E. Walker, M. Loik, D. Tissue, and J. C. Zak. Simulated precipitation on soil microbial processes and nitrogen dynamics in Big Bend National Park. Soil Ecology Society 9th Biannual Meeting, Palm Springs, CA May 2003.
22. Resinger, J. S., J. Sirotnak, and J. C. Zak. Microbial and soil nutrient dynamics associated with degraded grasslands in Big Bend National Park. Soil Ecology Society 9th Biannual Meeting, Palm Springs, CA May 2003.
23. Robertson, T., E. Walker, J. Zak, and D. Tissue. Plant responses to variation in timing and magnitude of precipitation in Big Bend National Park. Ecological Society of America Annual Meeting, Savannah, GA.
24. Sobek, E. A. and J. C. Zak. The functional capacity of desert soil fungi: from landscape to locale, to site. Soil Ecology Society 9th Biannual Meeting, Palm Springs, CA May 2003.

Awards and Honors

Jim Campbell, a Ph.D. graduate student with Dr. John Zak, won an award for his presentation at the biannual meeting of the Soil Ecology Society that was held at Argonne National Labs May Jim's talk was entitled: "Effects of Soil Chemistry on Distributions of Oligotrophic Bacteria Along an Elevational Gradient".

Lisa Patrick, a Ph.D. graduate student with Dr. David Tissue, was awarded an EPA STAR/GRO Fellowship to support her work at Big Bend National Park from September 2005 - August 2008.

Lisa Patrick was also awarded an ARCS scholarship to support her research efforts at Big Bend National Park from September 2005 to August 2007.

Conclusions

Plant Physiological Responses to Supplemental Watering

Plant carbon fixation responded positively to supplemental precipitation throughout the summer. Sotol, brown-spined prickly pear and side-oats grama plants in watered plots had increased rates of photosynthesis following water pulses in June and July in 2005. By September, only grasses in watered plots had higher rates of photosynthesis than plants in the control plots.

Soil respiration decreased in watered plots at the end of the summer (2005) when compared to controlled plots. Due to these increased rates of photosynthesis in grasses and decreased rates of daytime soil respiration, watered ecosystems were a sink for carbon in September, assimilating on average 31 mmol CO_2 m^{-2} ground area day^{-1}.

As a result of a 25% increase in summer precipitation, watered plots fixed eight times more CO_2 during a 24-hr period than control plots. In June and July, there were greater rates of transpiration for both grasses and shrubs in the watered plots. In September, similar rates of transpiration and soil water evaporation led to no treatment differences observed in ecosystem evapotranspiration, even though grasses transpired significantly more than shrubs.

Plant Growth and Dynamics

Dasylirion leiophyllum (Sotol) did show increased growth with increasing annual precipitation amounts, but no response to supplemental water treatment. Both *Bouteloua curtipendula* (side-oats grama) in 2002 (in winter supplemental water treatment) and *Opuntia phaeacantha* (brown spined prickly pear)in 2003 (in summer-winter supplemental water treatment) showed increased growth to supplemental water treatment but only for one year. Total species richness and total plant density increased significantly with increasing precipitation, but there was no response to supplemental water treatments that were applied in this study. When divided into supplemental groups, only grass and herb species richness and densities showed significant increases with increasing precipitation, but no significant increase due to supplemental water treatments.

Annual culm production and ANPP of *Bouteloua* was significantly greater in the winter water treatment for 2003 but not for 2004 and 2005. Only *Dasylirion* ANPP showed a positive response to increasing annual precipitation. There was no water addition effect on *Opuntia* or *Dasylirion* for annual leaf or pad production or for ANPP.

Microbial Dynamics and Functional Diversity

Across all years the ability of the soil bacteria in the community plots to use a variety of carbon compounds (substrate activity and substrate richness) was lower in the summer watered plots than activities observed in all other water treatments. Soil fungal activity was not altered by the additional water irrespective of time of water application or amount (summer and winter). The impacts of additional water on microbial functional diversity were strongly influenced by seasonal differences in precipitation frequency and amounts. Following a dry winter the application of additional winter moisture would increase bacterial functional diversity on carbon substrates. If additional moisture occurred during a dry summer, bacterial functional diversity was only increased relative to the control in plots that received both additional summer and winter precipitation. Moreover, impacts of additional moisture appear to be cumulative in that for most of the three years, bacterial functional diversity on carbon substrates was highest in the control plots.

Impacts of additional nitrogen on bacterial functional diversity on carbon substrates were significantly dependent upon precipitation amounts received at the high elevation Oak-Pine Forest but not at the Sotol Grasslands site. Increased nitrogen did not alter soil bacterial functional diversity on carbon substrates at the grasslands site. However, within the high elevation Oak-Pine Forest location, bacterial functional diversity was either reduced or enhanced depending upon previous moisture patterns with increasing amounts of nitrogen entering the soil system. During the summer increased nitrogen inputs following a drought period resulted in reduced bacterial functional diversity on carbon compounds. When nitrogen additions corresponded to a wet period, bacterial functional diversity on carbon substrates increased.

Fungal functional diversity on carbon substrates was not affected by increasing nitrogen input at either the Sotol Grasslands or high elevation Oak-Pine sites, irrespective of precipitation amounts. Additional nitrogen did increase the ability of the soil fungi in the Sotol Grasslands and at the high elevation Oak-Pine sites to use organic nitrogen substrates (functional diversity of fungi on nitrogen substrates). This priming effect occurred during the first two years of the study. The increase in organic nitrogen use was associated with amino acids and amides. Subsequently, as precipitation amounts decreased during the final years of the study, fungal functional diversity on nitrogen substrates decreased in plots receiving the 4X yearly amounts of nitrogen.

Soil Nutrient Dynamics

Changes in precipitation amounts and season of application did not significantly alter nutrient availability of either extractable NH4-N or NO3-N across years. However, within a particular year, depending upon previous moisture availability, soil nutrient pools were altered by additional soil moisture indicating a significant treatment by season interaction.

Lessons Learned

Greater amounts of summer precipitation may lead to short-term increased carbon uptake by this sotol grassland ecosystem. Increased summer precipitation in the Sotol Grassland delays their seasonal transition to a CO_2 source by enabling continued CO_2 uptake up by this ecosystem. Grasses, and not shrubs, exhibited a positive photosynthetic response to the 25% increase in summer precipitation one month after the last watering event. Because GCM precipitation scenarios include a >25% increase for Big Bend National Park, we predict that the Sotol Grasslands may begin to store soil carbon.

Grassland ecosystem along the Pine Canyon Watershed may respond to increased summer precipitation by increasing CO_2 fixation in late summer.

If grasslands in the Big Bend National Park continue to decline, the amount of CO_2 fixed across the landscape will decline as grasslands are converted to shrub dominated systems.
Annual precipitation in the Sotol Grasslands explained less than 15% of the variation in plant production and ANPP, clearly indicating that other factors such as soil chemistry (*e.g.* nitrogen), microbial dynamics, and plant phenology may have a great impact on productivity.

Although overall there were not significant increases in growth of the three plants studies over three years, there were still increases in plant production with increasing precipitation depending on the timing of the precipitation. *Dasylirion* showed a continuous increase in plant growth throughout the years. *Opuntia* response depended more on the timing with its phenology than with total precipitation. For *Bouteloua*, the greatest plant production followed a dry year after winter precipitation then decreased in subsequent, wetter years. Both *Opuntia* and *Bouteloua* had declines in leaf nitrogen for 2004, so soil nitrogen may also be more limited in a wetter year.

If precipitation in the southwestern United States increases as global models predict, then increased plant production of key dominant species in these Sotol-Grasslands may occur if nitrogen and other resources do not become limiting. This increase in plant production will also provide future challenges for the management of desert ecosystem. One challenge would be possible increases in fire frequency and intensity due to increased fuel loads, which would be especially important if a dry year follows a particularly wet year. Another challenge would be increased competition for other limited resources that may eventually lead to possible shifts in plant dominance in the grassland community.

Increased moisture and nitrogen input to these mid-elevation Sotol Grasslands will likely shift the systems to a fungal dominated one. Increase in fungal activity may lead to increased litter decomposition and increased fungal activity may result in an accelerated decline in soil organic matter with increased precipitation.

Changes in precipitation frequency and amounts do not appear to either increase or decrease soil nutrient pools on a permanent basis. However, additional moisture during the winter or summer will likely influence nutrient availability during periods of active plant growth as demand by the vegetation will be greater during those intervals. Impacts of increased precipitation on available-N may be delayed several months because the effect is dependent on the short-term soil microbial response to precipitation frequency and amount.

The decline in fungal functional diversity on nitrogen substrates as inorganic nitrogen input increases in the Sotol Grasslands suggests that the system may be becoming nitrogen saturated.

Methods Employed and Project Personnel

Plant Physiological Responses

To determine if plant available water increased with watering treatment, plant water potential (Ψ) was measured in using a Scholander-type pressure chamber (3000 Series, Soil moisture Equipment Corp., Santa Barbara, CA, USA). – Conducted by Lisa Patrick and David Tissue

The time course of whole-plot CO_2 and H_2O exchange with an open path infrared gas analyzer (IRGA; LI-7500, LI-COR Inc., Lincoln, NE, USA) located inside a static, closed gas exchange system was measured using the system described by Huxman et al. (2004). Briefly, this system consists of a static chamber (1.5 m wide × 1.8 m long × 1.8 m tall) constructed of a PVC pipe frame covered by a clear polyethylene film. Similar systems have been successfully used previously to measure whole ecosystem CO_2 and H_2O fluxes in arid environments (Arnone and Obrist 2003; Jasoni et al. 2005). During each ecosystem flux measurement, the IRGA was interfaced with a laptop computer and placed in the plot adjacent to a tripod mounted with two 15 cm diameter fans to maximize chamber mixing. The chamber was then placed on top of the 3 m × 3 m rainfall-treatment or control plot in a designated location to encompass vegetation representative of the whole plot. Data collection began approximately 30 seconds after placement of the chamber to allow for adequate mixing within the chamber, and whole-plot CO_2 and H_2O fluxes were recorded for 90 seconds (thermocouples showed no appreciable warming inside the chamber). A 24-hr time course of ecosystem flux measurements was conducted from 1530_h on September 14, 2004 to 1400_h on September 15, 2004. Daytime net ecosystem exchange (NEE_{day}) of CO_2 and evapotranspiration (ET_{day}) were measured eight times from dawn to dusk (0730, 0900, 1030, 1200, 1400, 1530, 1700 and 2000_h). Nighttime net ecosystem exchange of CO_2 (NEE_{night}) and evapotranspiration (ET_{night}) were measured twice from dusk to dawn (1930 and 0730_h). Measurements of NEE_{night} and ET_{night} at 0430_h were estimated by averaging data measured at 1230_h and 0730_h. Ecosystem CO_2 and H_2O flux measurements throughout the day and night were used to estimate integrated total daily fluxes of CO_2 (NEE_{total}) and H_2O (ET_{total}) for each plot. – Conducted by Lisa Patrick and David Tissue.

Leaf photosynthetic gas exchange [net assimilation rate (*A*), transpiration during the day (E_{day}), and stomatal conductance (g_s)] were measured with a portable open-flow gas exchange system (Model LI-6400, LI-COR Inc.) on recently mature leaves on one plant per functional type per plot (grasses -*Bouteloua curtipendula* and shrubs -*Dasylirion leiophyllum*). The same leaves on each plant were repeatedly measured throughout the experimental period. Measurements were taken in the morning when gas exchange was at its maximum rate (0700 to 0930_h, based on preliminary measurements). Leaf-to-air vapor pressure deficit (vpd), air temperature, and CO_2 concentration (370 $\mu mol\ mol^{-1}$) of the cuvette were set to ambient environmental values for each measurement period and maintained constant for all measurements across plots. Irradiance (*Q*) was set to saturating light conditions (2000 $\mu mol\ m^{-2}\ s^{-1}$). Data were logged five times for each leaf and then averaged for each plant to be used as a statistical unit. - Conducted by Lisa Patrick and David Tissue

Measurements of daytime soil CO_2 efflux (R_{sday}) were made with an EGM-4 Environmental Gas Monitor connected to a USASRC-1 Soil Respiration Chamber (PP Systems, Amesbury, MA, USA). Within this closed system, the CO_2 concentration was measured every 8 seconds for a period of 1 minute and a quadratic equation was fitted to the relationship between the increasing CO_2 concentration and elapsed time to calculate a rate of soil CO_2 efflux. - Conducted by Lisa Patrick and David Tissue

Growth Measurements

The timing of growth measurements (leaf area and leaf production) varied with each species. For *Opuntia phaeacantha*, 3-5 pads were measured once a month for three years in each plot using a string to determine pad circumference (cm) and digital calipers to measure pad width (mm). Using pad circumference, the pad area ($A= \pi r^2$ cm^2) was calculated since previous data showed this to be more complete than only measuring the diameter. Pad volume was also calculated (V= A x width; cm^3). The total number of pads were counted during each measurement time. Total pad area was estimated by totaling the pad areas of all pads measured. Any new pads were numbered and added to the monthly measurements. The health status (*e.g.* herbivory, pad mortality, and pad damage) of the plants as well as any flower, pad, or fruit formation was also noted. *Dasylirion leiophyllum* growth measurements were made four times per year, once during each season for three years on this slow-growing species. The total number of leaves and new leaf production per individual were recorded during each measurement time. Ten to twenty random leaves were measured per individual for length (cm) and width (cm), which were used to calculate average leaf area (A=1/2 x length x width; cm^2) per leaf. Total leaf area per individual was estimated by multiplying the total number of leaves per individual with the average leaf area for that individual (Smith and Knapp 2001). The health status (*e.g.* herbivory, leaf mortality, and leaf damage) of the plants as well as any flower or fruit formation was also noted during measurement times. *Bouteloua curtipendula* growth measurements were conducted at least once a month beginning in early summer when the plants became active and continued until dormancy in November or December, for three years. The total number of shoots/leaves and the total number of culms were counted for each individual.

Ten to twenty leaves of each individual were measured for length (cm) and width (mm) to calculate estimated average leaf area (A=1/2 x length x width; cm^2). Total leaf area per individual was estimated by multiplying the total number of leaves with the average leaf area of each individual (Smith and Knapp 2001). The health status (*e.g.* herbivory, leaf mortality, and leaf damage) of the plants was also noted. - Conducted by Traesha Robertson and Natasja Van Gesteel

Plant Biomass Measurements.

Aboveground net primary productivity (NPP) and annual aboveground net primary productivity (ANPP) were determined non-destructively. Off-plot samples of each species were collected to develop allometric regressions between field estimated leaf area and biomass (Retta et al. 2000; Smith and Knapp 2001). NPP was estimated for each month by subtracting the month's total plant biomass (vegetative and reproductive) from the previous month. This was done for each month and then totaled at the end of each year to obtain aboveground ANPP for that year. Only positive increments were used since it is generally difficult to determine whether any negative increment values (declines in biomass) are due to herbivory and senescence or to human error (Huenneke et al. 2001). The ANPP values are still an underestimate of productivity since belowground productivity was not determined (Huenneke et al. 2001). - Conducted by Traesha Robertson and Natasja Van Gesteel

Leaf C:N measurements.

Samples for analysis of leaf nitrogen and carbon were taken in September 2003 and 2004 from each of the plant species. They were dried and ground, then analyzed at Texas Tech University using a CN analyzer (NCS 2500, Carlo Erba Inc, Milan, IT) to determine leaf percentage of nitrogen and carbon and to calculate C:N ratios. – Conducted by Traesha Robertson and Natasja Van Gesteel

Soil Moisture Measurements. Volumetric soil moisture content was measured in 2004 using ECH_2O-10 dielectric aquameter probes (Decagon Devices, Pullman, WA, USA). One probe was placed in each plot at a soil depth of 15 cm. Measurements were logged every 2 hours on Em5 data loggers and averaged for the 24 hr period. – Conducted by John Zak, Colin Bell, Heath Grizzle, and Natasja Van Gestell

Microbial Dynamics and Functional Diversity

Microbial functional diversity on carbon substrate utilization was assessed for all water and nitrogen treatments for both bacteria and fungi by using the Biolog GN2 96 well microtiter plates to observe bacterial functional diversity, and the Biolog SFN2 microtiter plates to observe fungal functional diversity (Zak et al 1994) and Zak and Sobek (2003). Plates were inoculated from either soil dilutions (bacteria) or soil organic matter particles (fungi), incubated at 25º C, and read at 72 and 120 hours after incubation. Functional diversity is quantified as the total Substrate Activity (the total optical density for each replicate microtiter plate) and Substrate Richness (total number of substrates used). – Conducted by Colin Bell, Heath Grizzle, Amber Nagy, Jennifer Resinger, Natasja Van Gestell, and John Zak.

Microbial Biomass Carbon

Seasonal patterns and impacts of water and nitrogen treatments on the amounts of microbial biomass carbon (µg/g dry wt of soil) were assessed using the chloroform fumigation technique described by Vance et al (1987). Briefly soils were extracted with a 0.5M K_2SO_4 solution. The K_2SO_4 solution was filtered and frozen until the amount of dissolved carbon analyzed. Prior to analysis, samples were thawed and refiltered. The amount of carbon was measured using the spectophotometric procedure described by Nunnan et al (1998). – Conducted by Colin Bell, Heath Grizzle, Amber Nagy, Jennifer Resinger, Natasja Van Gestell, and John Zak.

Microbial Functional Diversity on Nitrogen Substrates

Soil Organic Matter (SOM) particles were wet sieved from soil samples obtained from the nitrogen plots in April and August of each year onto 500 and 250µm sieves. SOM particles from the 250µm sieve were used to inoculate BIOLOG PM3 microtiter plates, which contain 95 different nitrogen substrates. To inoculate the PM3 microtiter plates, 1 g/L of dextrose was added to the inoculum of 0.2% water agar plus 50 mg of sieved SOMs. Plates were incubated at 25 C and read AT 120 hours with an automatic plate reader (Grizzle and Zak 2006)**.** Two metrics of fungal functional diversity were obtained from this procedure: 1) Substrate Activity (the sum of the optical density of all 95 wells on a microtiter plate) and Substrate Richness (Number of nitrogen compounds that would support growth). – Conducted by Heath Grizzle, Colin bell, Jeb Clark, Natasja Van Gestell, and John Zak.

Financial Accounting

All funds were allocated as specified in the initial budget.

Salaries

During the summer months the following graduate students were supported at some point during the duration of the project: Colin Bell, Jim Campbell, Heath Grizzle, Jeb Clark, Amber Nagy, Lisa Patrick, Jennifer Resinger, and Traesha Robertson.

Travel:

Funds were used to support travel to field locations and for some of the meetings listed in the presentation section of this report

Operating Expenses

Funds were used to support all field and laboratory efforts as designated in the original proposal. Field items included installation and upkeep of Decagon Soil Moisture probes and loggers, Onset Hobo soil temperature probes, purchase of three water storage tanks, and expendable items for sampling of plant and soil materials. Laboratory expenses included purchasing of Biolog plates for microbial functional diversity assessment, chemicals, pipette tips and costs of NO_3-N measurements by A&L Laboratory in Lubbock.

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