Nematode Herbivory as a Mechanism Behind the Influence of Precipitation on the Partitioning of Net Primary Production between Above and Belowground Components

by

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ABSTRACT

Aboveground net primary production (ANPP) and belowground net primary production (BNPP) may not be influenced equally by the same factors in arid grasslands. Precipitation is known to affect ANPP and BNPP, while soil fauna such as nematodes affect the BNPP through herbivory and predation. This study on black grama grass (*Bouteloua eriopoda*) in the Chihuahuan Desert investigates the effects of precipitation and nematode presence or absence on net primary production (NPP) as well as the partitioning between the aboveground and belowground components, in this case, the fraction of total net primary production occurring belowground (fBNPP). I used a factorial experiment to investigate the effects of both precipitation and nematode presence on the components of NPP. I used rainout shelters and an irrigation system to alter precipitation totals, while I used defaunated and re-inoculated soil for the nematode treatments. Precipitation treatment and seasonal soil moisture had no effect on the BNPP and a nonsignificant positive effect on the ANPP. The fBNPP decreased with increasing precipitation and seasonal soil moisture, though without a significant effect. No predator nematodes were found in any of the microcosms at the end of the experiment, though other functional groups of nematodes, including herbivores, were found in the microcosms. Total nematode numbers did not vary significantly between nematode treatments, indicating that the inoculation process did not last for the whole experiment or that nematodes had little plant material to eat and resulted in low population density. Nematode presence did not affect the BNPP, ANPP, or the fBNPP. There were no significant interactions between precipitation and nematode treatment. The results are inconclusive, possibly as a result of ecosystem trends during an unusually high

precipitation year, as well as the very low NPP values in the experiment that correlated with low nematode community numbers.

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INTRODUCTION

Grassland ecosystems cover 25-30% of Earth’s land area, and consist of about 5% of net primary productivity (Sims et al. 1978, Field et al. 1998, Hui and Jackson 2006). Overall net primary production (NPP) in grasslands is low, though a large percentage of grassland NPP occurs belowground and an understanding of grassland NPP requires an understanding of the belowground component of NPP (Hui and Jackson 2006). Investigation of the mechanisms that influence NPP and the partitioning of NPP in grasslands allows for a better understanding of these ecosystems. A main influence on aboveground net primary production (ANPP) in grassland ecosystems is precipitation, with higher ANPP in more mesic grasslands as compared to more arid grasslands (Hui and Jackson 2006, Sala et al. 2012). The majority of grassland NPP occurs belowground (BNPP), though many estimates of NPP only consider the aboveground component (Hui and Jackson 2006, Kang et al. 2013). Arid grassland NPP has a large belowground component, indicating that the ANPP should not be the only component of grassland NPP that is studied, and the mechanisms influencing the BNPP need to be considered as well.

The factors influencing ANPP have been well-studied (Noy-Meir 1973, Sala et al. 2012). In arid grasslands, ANPP is mainly influenced by precipitation, with little effect of other factors such as temperature (Noy-Meir 1973, Sala et al. 2012, Kang et al. 2013). Precipitation is the limiting factor in these ecosystems and as a result, greater precipitation leads to increased ANPP (Sala et al. 2012, Kang et al. 2013). In addition, a strong positive correlation between ANPP and mean annual precipitation in arid grasslands indicates that ANPP is influenced by the average amount of precipitation received (Sala et al. 2012, Kang et al. 2013). Yearly precipitation within one grassland can also have an effect on ANPP; higher than average precipitation leads to increased ANPP for that year (Wilcox et al. 2015). On the whole, precipitation has a strong impact on ANPP in grassland ecosystems.

Studies on primary productivity typically focus on ANPP, rather than including both the aboveground and belowground components of NPP (Hui and Jackson 2006). ANPP tends to be studied more than BNPP in part because of a difficulty in measuring belowground biomass; different sampling methods result in differences in BNPP measurements (Hui and Jackson 2006). Though the most frequently studied component of NPP is the ANPP, this represents less than half of arid grassland primary productivity (Hui and Jackson 2006, Kang et al. 2013). Studies of belowground biomass in grasslands have shown that it increases with greater mean annual precipitation in a similar manner to the aboveground biomass (Kang et al. 2013). In addition, BNPP is influenced by soil biota that affect root growth and nutrient uptake (van der Putten et al. 2013). Some soil organisms influence plant nutrient uptake by providing nutrients such as nitrogen to the plants (van der Putten et al. 2013). Other soil organisms, such as some nematode species, can affect root growth by consuming the roots (Ferris 2010). These belowground factors affect the BNPP, suggesting that the main factors that influence BNPP may be different than the factors that influence ANPP.

One way to better understand the controls behind ANPP and BNPP and how they differ is to investigate the fraction of the total primary production that occurs belowground. Is this fraction, or the fBNPP, influenced by the same factors that determine ANPP and BNPP? The fBNPP could be important in understanding ecosystem processes such as carbon cycling (Hui and Jackson 2006). Though both ANPP and BNPP respond to some of the same factors, the fBNPP can also change, indicating that ANPP and BNPP may not be equally affected by factors such as precipitation.

Soil fauna such as nematodes can affect the BNPP in a different way than the ANPP. Nematodes are an abundant phylum of belowground fauna that can affect plant growth (Vandegehuchte et al. 2015). Interactions between nematodes and plants are both direct, such as with herbivory of roots, and indirect, such as predation of herbivorous nematodes by predator nematodes (van der Putten et al. 2013). Nematodes have a variety of feeding habits, including herbivory and predation, and therefore occupy multiple trophic levels (Ferris 2010). Herbivorous nematodes directly affect BNPP (Vandegehuchte et al. 2015). Other nematode species in higher trophic levels are predators and consume organisms such as bacteria and the herbivorous nematodes (Ferris 2010). This results in a trophic structure with predation on herbivorous nematodes leading to changes in plant growth (Ferris 2010). Predation on herbivorous nematodes will result in an indirect effect on plants, as fewer herbivorous nematodes consuming plant roots allows the plants to grow more.

Nematodes are aquatic and enter an inactive state called anhydrobiosis if they begin to dry out (Freckman et al. 1987). Predatory nematodes tend to be larger than herbivorous nematodes, which could result in a change to the trophic cascade if there is not enough water for the larger predatory nematodes (Vandegehuchte et al. 2015). Therefore, nematode trophic levels respond in different ways to changes in precipitation, with predators expected to be more sensitive to drought than herbivores. Predatory nematodes control herbivorous nematode populations, but if the predatory nematode populations enter anhydrobiosis before the herbivores, then the herbivores will not have that trophic level pressure (Freckman et al. 1987, Ferris 2010). Nematode trophic levels can also respond differently to precipitation pulse size and length, which also affects the trophic structure (Schwinning and Sala 2004). The result of this variation in nematode sensitivity to changes in precipitation pulses is expected to be a trophic cascade with less predation on herbivorous nematodes and more herbivory in environments with lower precipitation.

To better understand the effects of precipitation and nematode presence on NPP, I have three research questions: (1) What is the effect of precipitation on the BNPP, ANPP, and fBNPP? (2) What is the effect of nematode presence on the BNPP, ANPP, and fBNPP? (3) What is the interactive effect of nematode presence and precipitation? To test these questions, I used a factorial combination of both the effect of precipitation manipulation and nematode presence/absence on black grama grass (*Bouteloua eriopoda*) growth in an arid grassland in the Chihuahuan Desert. I used two drought treatments (with 20% and 50% of ambient precipitation) and two irrigation treatments (with 150% and 180% of ambient precipitation) to test the effects of precipitation, as well as a nematode treatment without nematodes (defaunated) and one with the native nematode community (inoculated). This allowed me to investigate the effects that precipitation, nematode inoculation, and the interaction of the two have on the partitioning of NPP into aboveground and belowground components, as well as on the fBNPP. I predict that ANPP and BNPP will increase as precipitation increases, but that fBNPP will decrease as precipitation increases (Figure 1). I also predict that nematode presence will decrease BNPP and fBNPP (Figure 1). I expect that the interaction of the two variables will result in the defaunated nematode treatment having greater BNPP, ANPP, and fBNPP than the inoculated nematode treatment in all precipitation treatments, with the effect of nematode treatment becoming larger as precipitation increases.

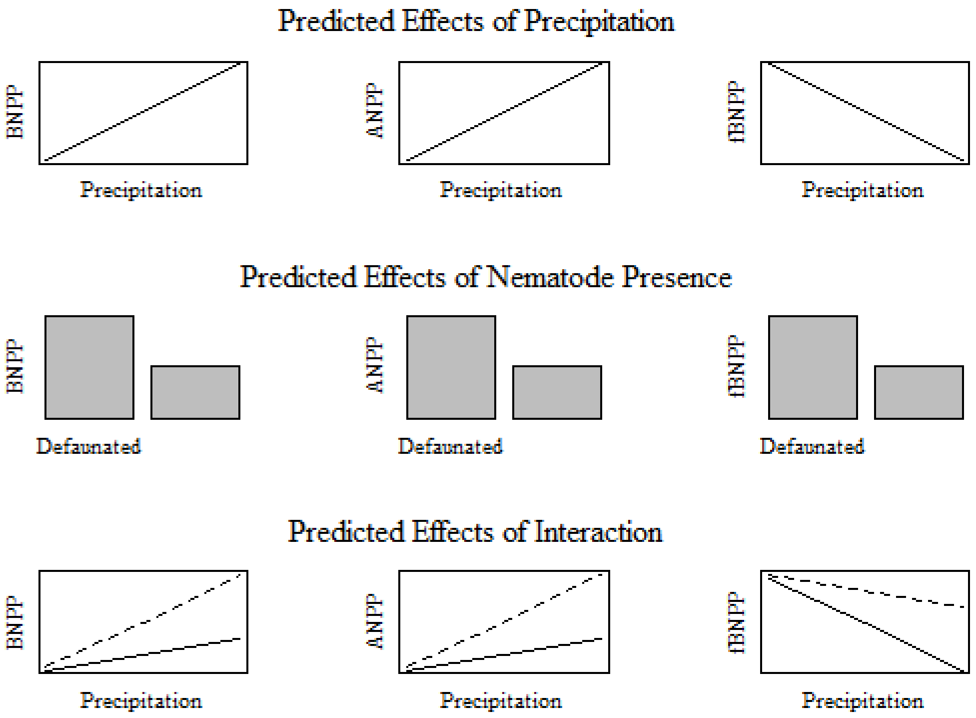

Figure 1. Predicted effects of precipitation, nematode presence, and their interaction on belowground net primary production (BNPP), aboveground net primary production (ANPP), and the fraction of belowground net primary production (fBNPP). In the last row, the dotted line indicates defaunated nematode treatment and solid line indicates inoculated nematode treatment.

METHODS

Location

This study took place at the Jornada Long Term Ecological Research (LTER) site in the northern Chihuahuan Desert in New Mexico. The Jornada LTER is located northeast of Las Cruces, New Mexico. The mean annual precipitation is 245 mm and the mean annual temperature is 14°C, with summer temperatures reaching 36°C (Havstad et al. 2006). Total precipitation in 2017 was 289.8 mm; growing season (July-October) precipitation was 172.0 mm (Jornada LTER). The rainy season in the northern Chihuahuan Desert is from July to October, when the desert receives a majority of the annual rainfall (Freckman et al. 1987). Black grama grass (*Bouteloua eriopoda*) dominates grasslands at the Jornada (Havstad et al. 2006). Black grama grasslands are composed primarily of black grama, but also include other grasses such as three-awns (*Aristida spp).* and dropseeds (*Sporobolus spp.)*, as well as shrubs such as mesquite (*Prosopis glandulosa*) which can be present in the grasslands as well (Havstad et al. 2006).

Experimental Design

I used a factorial experimental design to test the effects of precipitation manipulation and nematode presence or absence on BNPP, ANPP, and fBNPP within the experimental plots. I used precipitation treatments to increase or decrease the percentage of total precipitation received compared to the ambient, and I used black grama grown in treated soil in microcosms (see description below) within the precipitation treatments to test the effect of nematode presence or absence. The precipitation and nematode treatments were combined to test for any interaction effects.

The field site for this experiment at the Jornada LTER was established for a study on “Water Availability Controls on Above-Belowground Productivity Partitioning” (Sala and Wall, unpublished). There are 40 plots in total for the “Water Availability Controls” study, with a subset of 25 used in this experiment. The plots (2.5 by 5 meters) for the experiment were chosen randomly in a grass-dominated pasture at the Jornada LTER.

Precipitation Treatments

I used precipitation manipulation treatments to reduce or increase the percentage of total precipitation throughout the experiment, following the design of Gherardi and Sala (2013). Rainout shelters reduced the percentage of precipitation. Clear acrylic shingles formed the roof of each shelter and reduced the percentage of precipitation by either 50% or 80% (n=5 plots in each treatment). An irrigation system connected the rainout shelters to irrigation plots and increased the precipitation by either 50% or 80% (n=5 plots in each treatment). The percentage of increased irrigation corresponded with the equivalent reduction of precipitation from the rainout shelters and was therefore a precipitation supplement. The irrigation system consisted of a collection bin at the shelters for runoff water from the shingles and a pump facilitating water transport through PVC pipe running to a sprinkler system at the irrigation plot. A float switch activated the pump when the water level in the bins increased during each precipitation event. Control plots received no precipitation manipulation (n=5 plots).

Soil Treatments

I collected soil from an area adjacent to the plots in the field experiment. After I collected the soil, I sieved it to remove roots and rocks, and then stored the soil at room temperature until I defaunated it. I defaunated all the soil to kill all nematodes before I placed it in the microcosms (Franco et al. 2017). Half of the microcosms were then inoculated with nematodes after the microcosms were placed in the field. This experiment used the soil defaunation process from Franco et al. (2017), which successfully kills more than 98% of nematodes in the soil.

The first step to defaunation involved placing soil 5 cm deep in aluminum trays and soaking the soil until it reached the saturation point. To determine the saturation point, I poked several holes in the bottom of the trays so that water could drip through when the soil became saturated. Immediately after wetting the soil, I placed it in a refrigerator at 4°C for 24 hours. I then placed the soil in a drying oven at 65°C for 48 hours. I stored all defaunated soil in containers that I cleaned with 70% ethanol, to ensure that no nematodes remained in the containers.

All microcosms were inoculated with either a microbial or nematode and microbial solution after the microcosms were placed in the field. The nematode extraction and inoculation processes took place in collaboration with the Wall lab at Colorado State University. The two solutions of soil biota were obtained from a 13.5 kg subsample of the soil collected for defaunation and represented the nematode community at the experimental site. This sample represented the weight of soil in the top 5 cm of the 25 microcosms receiving the nematode inoculation. The soil sample was kept refrigerated at 4°C until nematodes were extracted. Nematodes were extracted from the soil sample with the use of a Baermann funnel technique, in which nematodes are flushed out the bottom of a soil sample (Hooper 1970). For this experiment, 135 100g soil samples were used in the funnels and the nematode water solution was collected after 3 days (Andre Franco, personal communication). The water solution containing the nematodes that was collected from each funnel was then allowed to sit for two hours until the nematodes settled at the bottom. The nematode inoculation solution was obtained from the bottom of the water from the Baermann funnels. Nematodes in the inoculation solution were counted by the Wall lab with a microscope (Olympus CKX41, 200X magnification) and identified by functional group (Yeates et al. 1993). The microbial solution used in the defaunated treatments was taken from the top of the water solution. It contained no nematodes—determined by looking at subsamples of the water solution—but contained other native microbes from the soil; these microbes were not identified but were similar between the two nematode treatments as it was a non-sterile water solution taken from the soil (Andre Franco, personal communication). Each microcosm was inoculated with 10 mL of one of the solutions one day after the microcosms were placed in the field.

Microcosm Design

The experiment consisted of soil microcosms (*n*=50) with black grama seedlings in one of the two nematode treatments that were placed in the field in one of the five precipitation treatments. Ten microcosms were placed in each precipitation treatment with two per plot, five with nematodes and five without nematodes. A microcosm experiment allowed for manipulation of soil variables to control for various elements in the field (Bruckner et al. 1995). Each microcosm consisted of a 20 cm tall, 10 cm (4-

inch) diameter PVC pipe filled with soil. I filled the microcosms with soil and planted them with black grama seeds and placed the microcosms in a greenhouse for two weeks to allow the seedlings to germinate before being placed in the field. I watered the seedlings in the greenhouse regularly. The microcosms were placed in the field in July 2017 and collected in October 2017.

To grow the seedlings, I put the defaunated soil in the PVC microcosms, which were cleaned with 70% ethanol prior to use. The bottom of each microcosm was covered in plastic wrap until I placed the microcosms in the field. I filled each microcosm with 2.15 kg of soil and watered them all to saturation (300 mL) before planting the seeds. To begin with, 20 black grama seeds were planted in each microcosm. I left the microcosms in a greenhouse for two weeks to allow the black grama seedlings to grow in an environment without nematodes before placement in the field; once the microcosms were placed in the field, nematodes on wind-blown dust particles could contaminate the defaunated microcosms.

The microcosms with seedlings were placed in the field in each of the precipitation treatments. Two soil cores were removed from each of the plots (*n*=25), one each for the defaunated and the inoculated microcosms that were put in each plot. Half the microcosms were inoculated with a nematode and microbial solution, while the other half were inoculated with only a microbial solution, to ensure that the soil biota besides nematodes were similar between the two treatments and that the inoculation process did not affect the results.

After one day in the field, I could no longer see many of the seedlings in the microcosms, a possible consequence of aboveground herbivory. Cages (about 30 cm tall by 13 cm in diameter) made of wire mesh placed over each microcosm prevented further aboveground herbivory. After a failed seedling transplant attempt, a second batch of black grama seeds were planted in the microcosms after the addition of the cages. The new seeds were watered weekly to ensure that enough seedlings survived. Each week during September, I watered each microcosm with 50 mLs of water, regardless of precipitation treatment. For the analysis, I added this additional water to the total ambient precipitation.

Response Variables

At the end of the growing season in October, the seedlings began to senesce. At this time, all the microcosms were collected from the field for analysis. The microcosms contained mostly black grama seedlings from the second time that seeds were planted, though several forbs grew in the microcosms, as well as 8 seedlings from the first time that I planted the black grama. Aboveground biomass and soil containing belowground biomass were collected separately for ease of analysis. Aboveground biomass was clipped out of each microcosm. I then removed all soil from each microcosm and stored the soil in bags in a cooler to keep the nematodes alive until the soil nematode community could be analyzed. I removed a 200 g homogenized soil sample from each microcosm for nematode community analysis. I washed the remaining soil to collect all roots, following the methods of Lauenroth and Whitman (1971). I then dried the aboveground and belowground biomass in a drying oven at 60°C for two days. I weighed the belowground biomass and aboveground biomass to obtain the dry weights. I used these dry biomass weights as a measure of the belowground biomass and aboveground biomass. I calculated the BNPP and the ANPP by determining the area of each microcosm and dividing the biomass in the microcosm by the area to calculate grams per square meter; the biomass in the microcosms was for one growing season, so the NPP was measured as $g/m^2/yr$. The fraction of belowground net primary production for each individual sample was calculated as:

$$fBNPP = BNPP/(ANPP + BNPP)$$

Data on nematode populations by functional group in the microcosms was provided by the Wall lab at Colorado State University from the samples of soil taken from the microcosms collected at the end of the experiment.

Seasonal soil moisture data was collected from soil moisture sensors (Campbell Scientific CWS655 probes) placed in three random plots (not necessarily the same as the microcosms) in each precipitation treatment, for a total of 15 soil moisture sensors. The sensors recorded the volumetric water content (%) at 12 cm depth. Soil moisture data was recorded by a data logger (Campbell Scientific CR800 data logger) every half hour throughout the summer and collected for analysis at the end of September (July 1 – September 29).

Statistical Analysis

I conducted the data analyses using the statistics program R (R Core Team 2017). I used linear models and type II ANOVA tests on the data to investigate the effect of precipitation or seasonal soil moisture, nematode inoculation, and their interaction. Assumptions of normality were not met for the BNPP, ANPP, and total nematode numbers. A log transformation on the BNPP, ANPP, and total nematode numbers allowed assumptions of normality to be met. I removed one microcosm from the analysis because it was disturbed in the field. I combined both nematode treatments in the analysis of precipitation and soil moisture because I found no significant effect of nematode treatment. Seasonal soil moisture data was averaged between all sensors within the same precipitation treatment, with null data from disturbances of the sensors removed; this included data from four sensors that did not record any data the whole season. Data was averaged by day for the season, as well as averaged throughout the season to obtain mean soil moisture values. Mean seasonal soil moisture was compared to precipitation with a type II ANOVA test.

RESULTS

In addition to the total precipitation recorded for the growing season, a total of 29.85 mm of water was added to all of the microcosms. Water was added on eight separate occasions to all microcosms, regardless of precipitation treatment. With the additional water, the control treatment received a total of 201.8 mm of precipitation during the growing season in the experiment. Precipitation manipulations received a percentage of this amount. Soil moisture varied throughout the season in all treatments but increased more in the higher precipitation treatments (Figure 2a). Mean soil moisture was greater in the higher precipitation treatments; mean soil moisture and precipitation are positively correlated (Figure 2b; $F(1,47)= 164.24$, $p<0.01$). These data show the difference in soil moisture between the precipitation treatments.

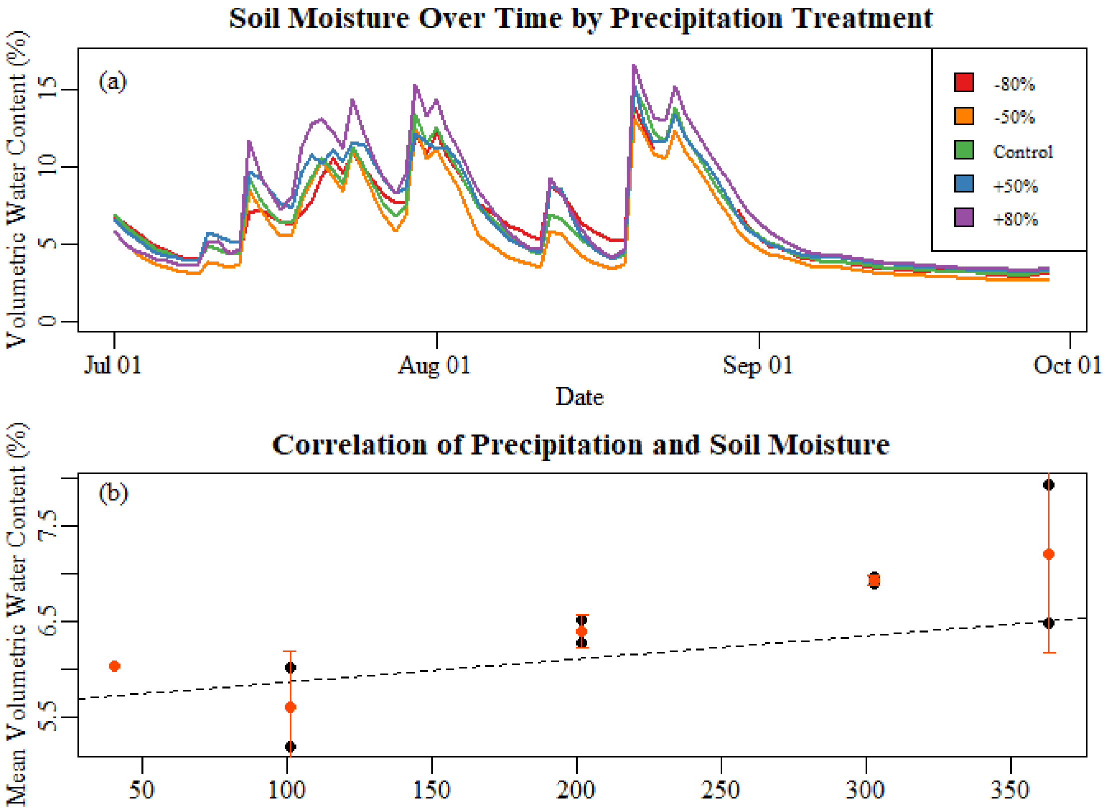

Figure 2. Average soil moisture by precipitation treatment over time and compared to total precipitation received. (a) Data represent averages for all soil moisture sensors in each precipitation treatment throughout the experiment. (b) Mean soil moisture for each sensor compared to the precipitation received; the -80% precipitation treatment shows average soil moisture across sensors because no single sensor recorded the whole season. Error bars show +/- one standard deviation from the mean for each precipitation treatment.

Effects of Precipitation

There was no effect of total precipitation on BNPP as compared to the control (Figure 3; F(1,47)= 0.2245, p=0.6378). No overall trend linked BNPP and precipitation. Mean BNPP in the control precipitation treatment was 40.7 g/m^2/yr, while mean BNPP in the irrigation treatments was 33.3 g/m^2/yr and 31.2 g/m^2/yr in the +50% and +80% treatments. The mean BNPP in the drought treatments was 31.9 g/m^2/yr and 31.7 g/m^2/yr for the -50% and -80% precipitation treatments. There was no trend in BNPP compared to seasonal soil moisture in the plots (Figure 4; F(1,47)=0.1803, p=0.6731).

The ANPP increased with increasing precipitation (Figure 5), though the effect of precipitation was not significant (F(1,47)=0.2014, p=0.6557). The mean ANPP in the control treatment was 18.4 g/m^2/yr and increased from 5.9 g/m^2/yr in the -80% precipitation treatment to 18.7 g/m^2/yr in the +80% precipitation treatment. The ANPP was also affected by seasonal soil moisture (Figure 6). The ANPP increased as soil moisture in the microcosms increased, though the trend was not significant (F(1,47)=0.354, p=0.5547).

The fBNPP decreased as precipitation increased (Figure 7). The overall relationship between the fBNPP and precipitation treatment was not significant (F(1,47)=0.9753, p=0.3284). The fBNPP decreased slightly as soil moisture in the microcosms increased (Figure 8; F(1,47)=1.471, p=0.2312). The fBNPP decreased from 0.841 (84.1%) in the -80% precipitation treatment to 0.760 (76.0%) in the +80% precipitation treatment.

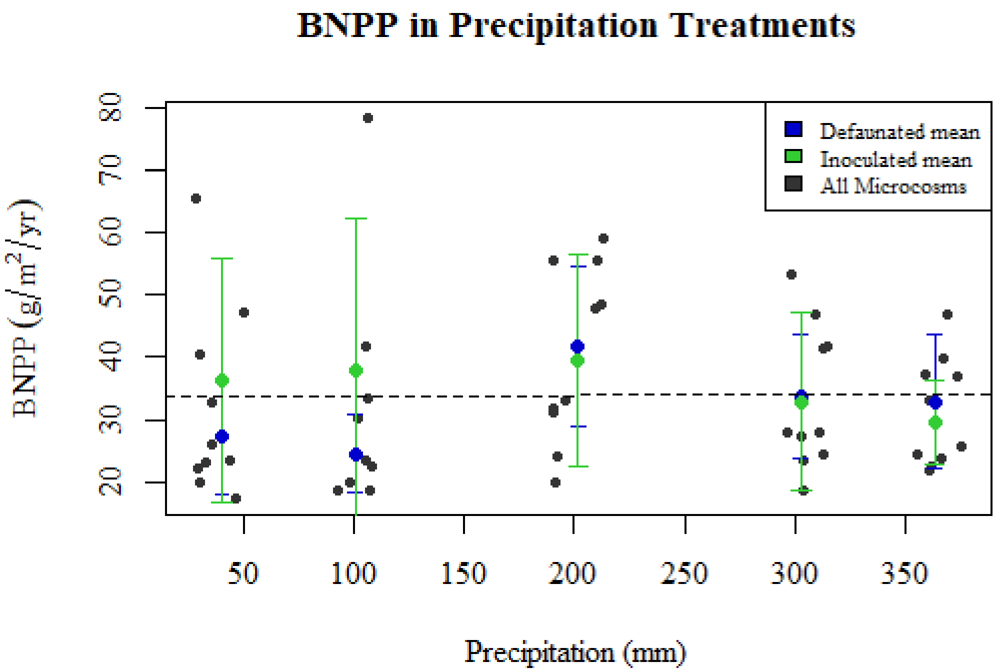

Figure 3. Effect of precipitation on BNPP. Individual data points shown in black (*n*=50), with average and error bars showing one standard deviation of each nematode treatment at each precipitation level shown in color. Trendline shows overall regression line for all microcosms.

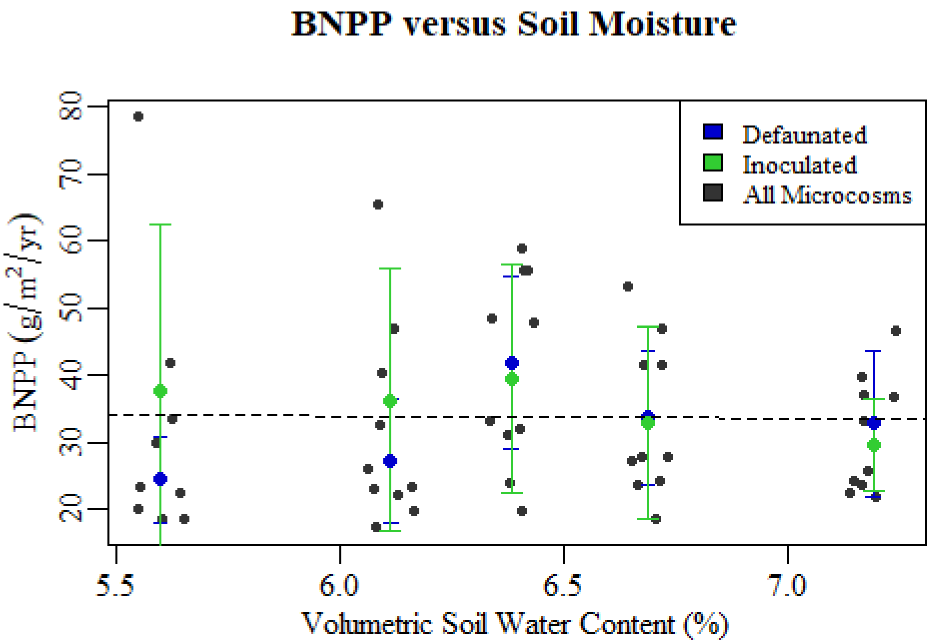

Figure 4. Effect of soil moisture on BNPP. Individual data points shown by nematode treatment (*n*=25 in each), with average and error bars showing one standard deviation of each nematode treatment at each precipitation level shown in color. Trendline shows overall regression line for all microcosms.

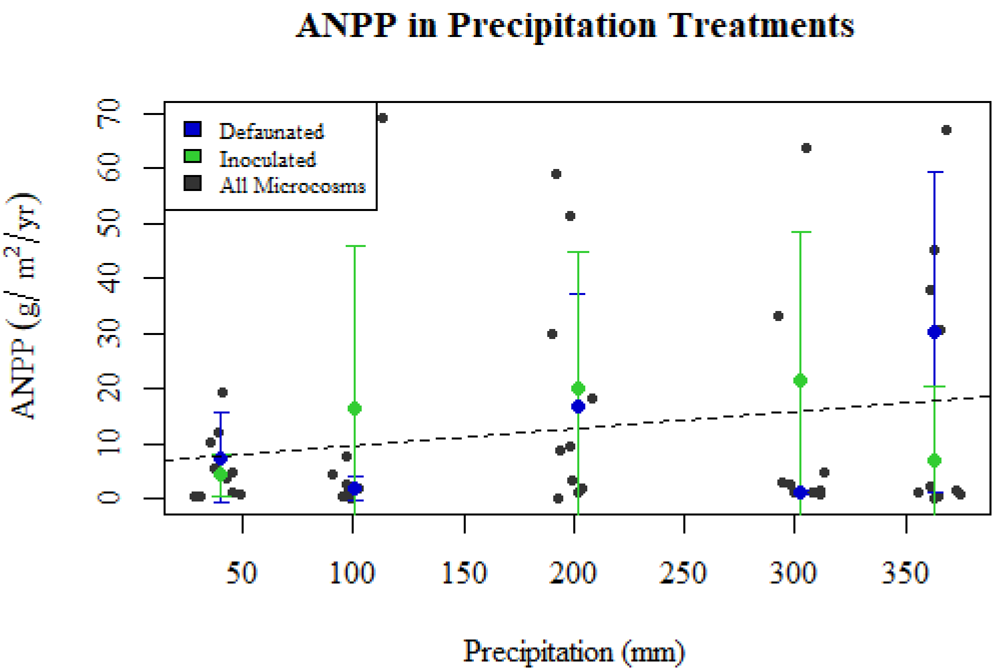

Figure 5. Effect of precipitation on ANPP. Individual data points shown in black (*n*=50), with average and error bars showing one standard deviation of each nematode treatment at each precipitation level shown in color. Trendline shows overall regression line for all microcosms.

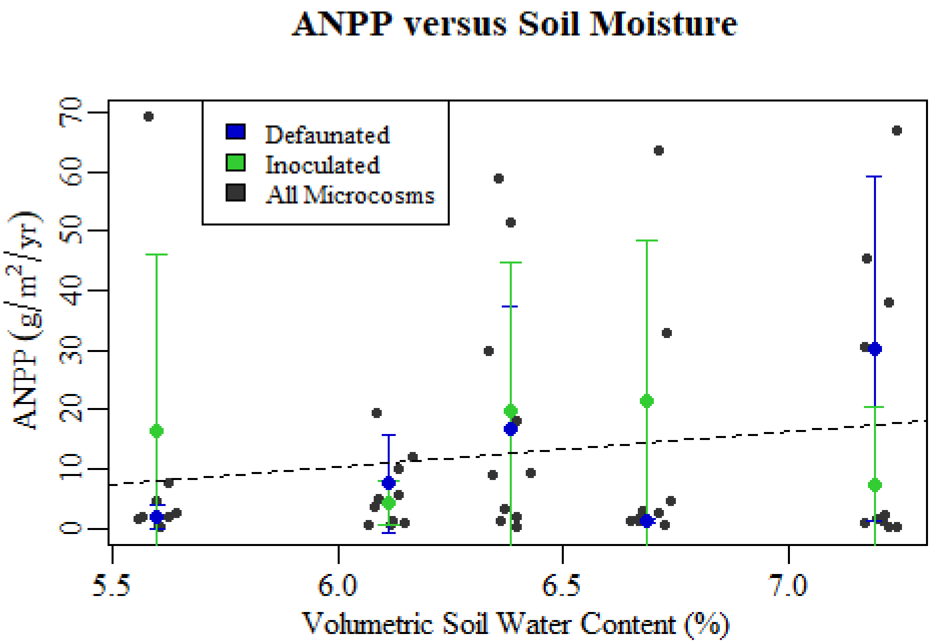

Figure 6. Effect of soil moisture on ANPP. Individual data points shown by nematode treatment (*n*=25 in each), with average and error bars showing one standard deviation of each nematode treatment at each precipitation level shown in color. Trendline shows overall regression line for all microcosms.

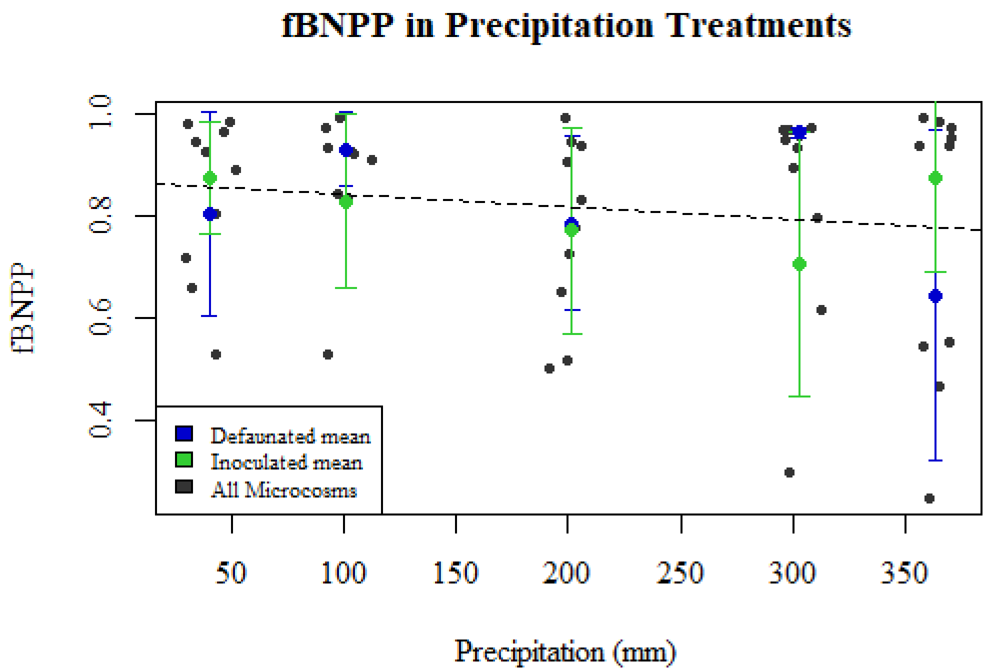

Figure 7. Effect of precipitation on fBNPP. Individual data points shown in black (*n*=50), with average and error bars showing one standard deviation of each nematode treatment at each precipitation level shown in color. Trendline shows overall regression line for all microcosms.

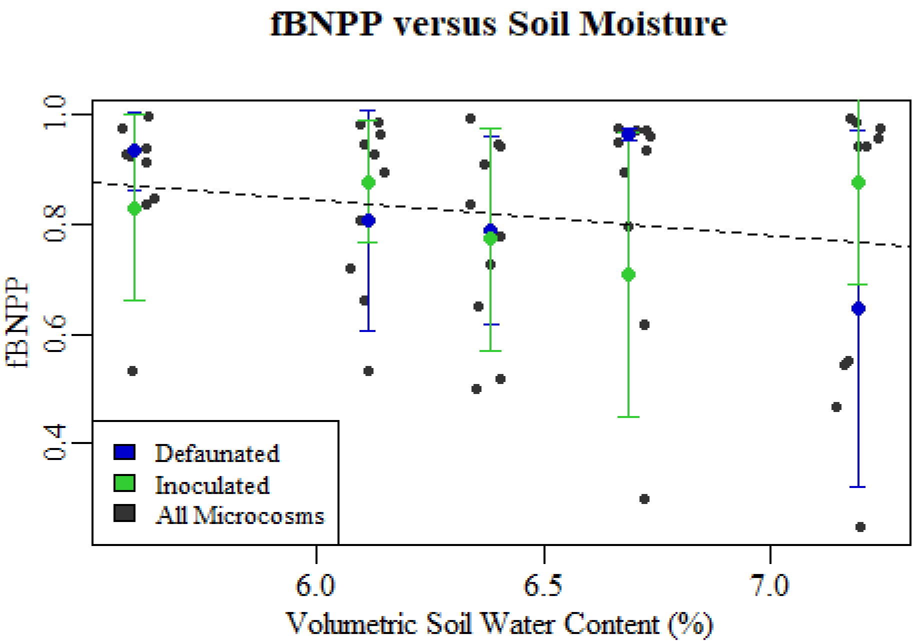

Figure 8. Effect of soil moisture on fBNPP. Individual data points shown by nematode treatment (*n*=25 in each), with average and error bars showing one standard deviation of each nematode treatment at each precipitation level shown in color. Trendline shows overall regression line for all microcosms.

Effects of Nematode Inoculation

Total nematode abundance was reduced in the defaunated treatment as compared to the inoculated treatment, though the effect was not significant (Figure 9; F(1,47)=0.0003, p=0.9868). All precipitation treatments were averaged together for the nematode abundance analysis because there was no significant difference between them. The mean number of total nematodes in the defaunated treatment was 645.9 individuals per kilogram of dry soil, while the mean number of nematodes in the inoculated treatment was 896.2 individuals per kilogram of dry soil. No predator nematodes were found in any of the microcosms. Other functional groups of nematodes (bacterivores, fungivores, omnivores, and plant parasites) were found in the microcosms, though not all functional groups were found in every microcosm.

The mean BNPP in the defaunated treatment was 32.3 g/m^2/yr, and the mean BNPP in the inoculated treatment was 35.2 g/m^2/yr. There was no significant difference in the treatments (Figure 10; F(1,47)=0.1964, p=0.6597). The defaunated treatment had a mean of 11.9 g/m^2/yr of ANPP, and the inoculated treatment had a mean of 13.9 g/m^2/yr of ANPP. The ANPP in different nematode treatments had no pattern (Figure 11; F(1,47)=0.4001, p=0.5301). There was also no clear pattern in the fBNPP in relation to nematode treatment (F(1,47)=0.0334, p=0.8557). The fBNPP was 82.3% in the defaunated treatment and 81.2% in the inoculated treatment (Figure 12).

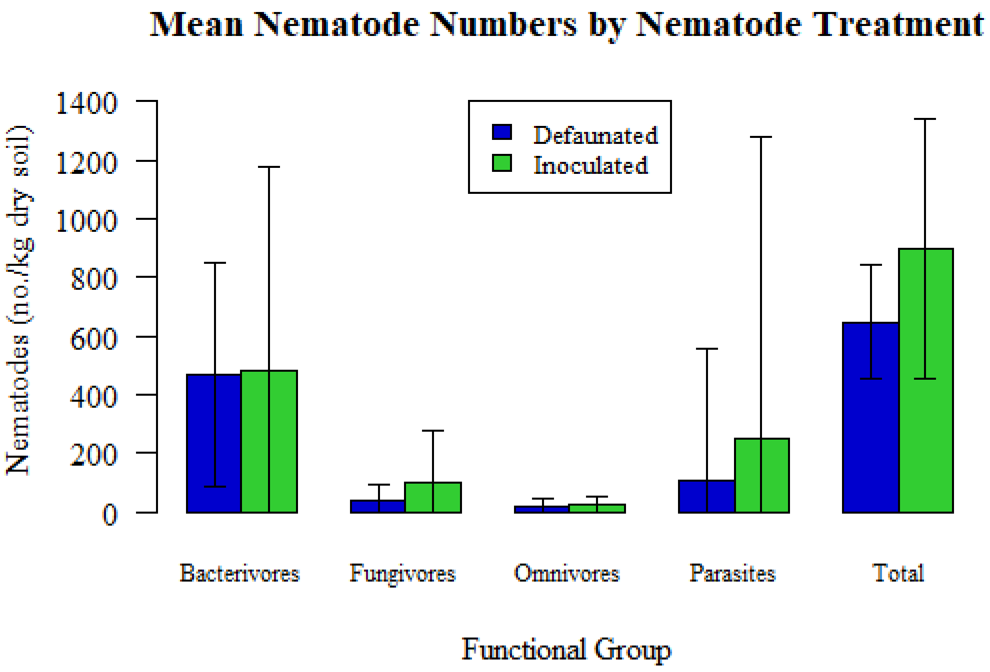

Figure 9. Mean numbers of nematodes per kilogram of dry soil in each functional group by nematode treatment. All precipitation treatments are averaged together. Predator nematodes are not shown because every value was zero. Some nematodes were not identified by functional group. Error bars show +/- one standard deviation from the mean for each treatment.

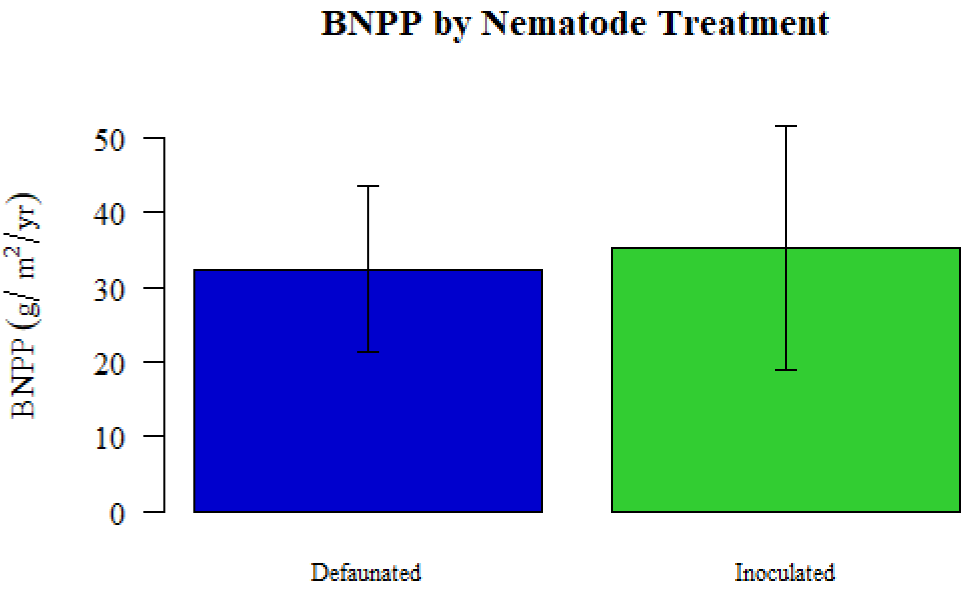

Figure 10. Mean BNPP by nematode treatment. Error bars show +/- one standard deviation from the mean for each treatment.

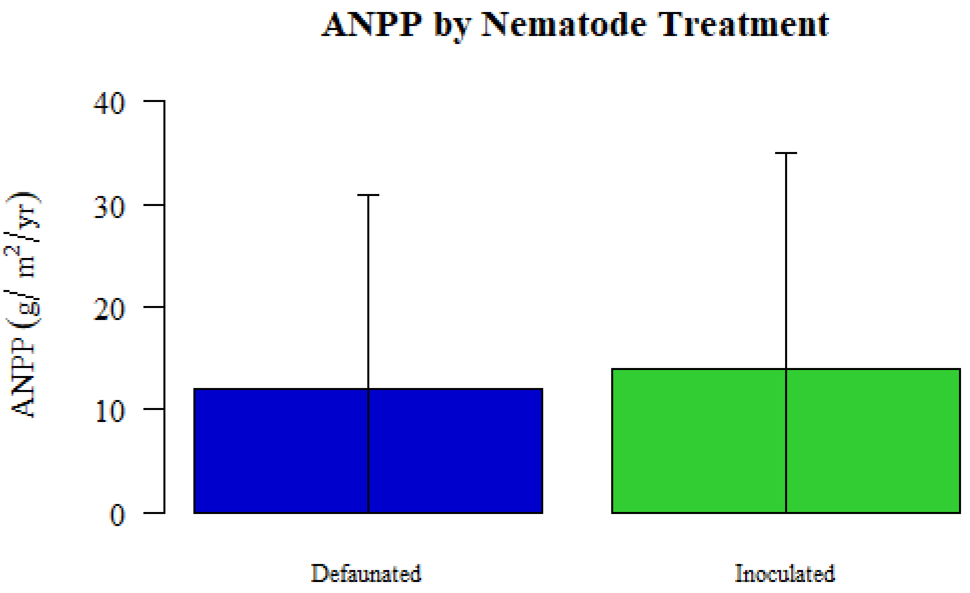

Figure 11. Mean ANPP by nematode treatment. Error bars show +/- one standard deviation from the mean for each treatment.

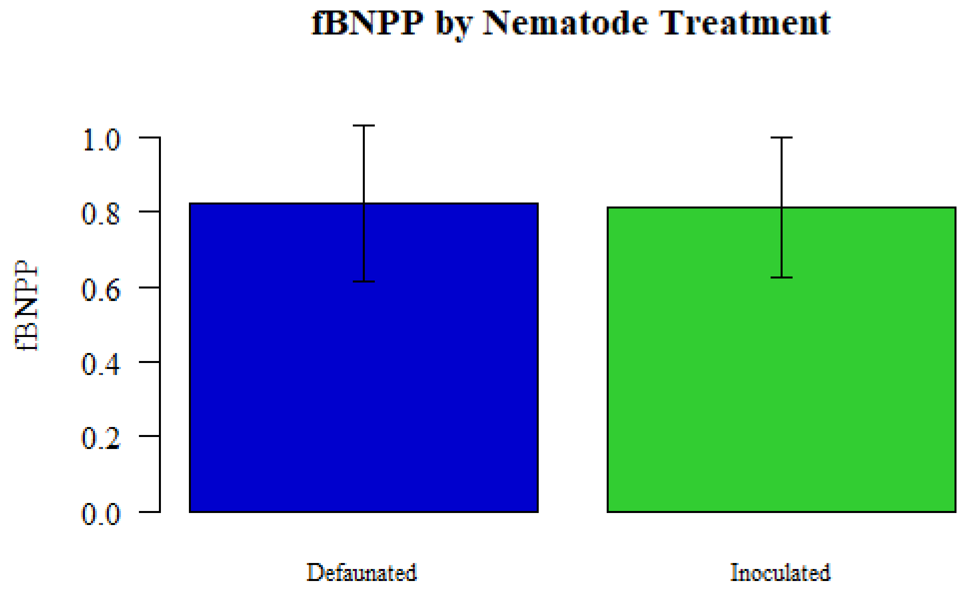

Figure 12. Mean fBNPP by nematode treatment. Error bars show +/- one standard deviation from the mean for each treatment.

Interaction Effects

The interaction between precipitation or seasonal soil moisture and nematode treatment was not significant for all three variables (BNPP, ANPP, and fBNPP). For the BNPP, F(1,45)=1.605 and p=0.2117 for the interaction effect of precipitation (Table 1). The inoculated treatment had a greater mean NPP in the two drought treatments, but lower NPP in the control and in the two irrigation treatments (Figure 3). The interaction with seasonal soil moisture was similar (Table 2; F(1,45)=1.5811 and p=0.2151).

For the ANPP, the interaction between precipitation treatment and nematode treatment was not significant (Table 3; F(1,45)=0.2510 and p=0.6118). There was no pattern for the difference between the two nematode treatments in the precipitation treatments. The defaunated treatment had a greater mean ANPP in the -80% and +80% precipitation treatments. The defaunated treatment had lower mean ANPP in the other three treatments (Figure 5). There was no interaction effect with seasonal soil moisture (Table 4; F(1,45)=1.7919 and p=0.1874).

For the fBNPP, F(1,45)=0.063 and p=0.802 for the interaction term (Table 5). The lack of a pattern in the interaction of nematode treatment and precipitation treatment was similar to the ANPP. The defaunated nematode treatment had a lower mean fBNPP in the -80% and +80% precipitation treatments, but a higher fBNPP in the other precipitation treatments (Figure 7). The interaction between soil moisture and nematode treatment was also not significant for the fBNPP (Table 6; F(1,45)=1.2214 and p=0.2750).

BNPP	Df	F value	Pr(>F)
Precipitation	1	0.2310	0.6331
Nematode	1	0.2031	0.6544
Precipitation x Nematode	1	1.6050	0.2117
Residuals	45		

Table 1. ANOVA table for a model of BNPP compared to the interaction between precipitation and nematode presence.

BNPP	Df	F value	Pr(>F)
Soil Moisture	1	0.1916	0.6637
Nematode	1	0.2076	0.6509
Soil Moisture x Nematode	1	1.5811	0.2151
Residuals	45		

Table 2. ANOVA table for a model of BNPP compared to the interaction between soil moisture and nematode presence.

ANPP	Df	F value	Pr(>F)
Precipitation	1	0.2053	0.6526
Nematode	1	0.3967	0.5320
Precipitation x Nematode	1	0.2510	0.6188
Residuals	45		

Table 3. ANOVA table for a model of ANPP compared to the interaction between precipitation and nematode presence.

ANPP	Df	F value	Pr(>F)
Soil Moisture	1	0.3802	0.5406
Nematode	1	0.4260	0.5173
Soil Moisture x Nematode	1	1.7919	0.1874
Residuals	45		

Table 4. ANOVA table for a model of ANPP compared to the interaction between soil moisture and nematode presence.

fBNPP	Df	F value	Pr(>F)
Precipitation	1	0.9423	0.3369
Nematode	1	0.0391	0.8441
Precipitation x Nematode	1	0.0633	0.8024
Residuals	45		

Table 5. ANOVA table for a model of fBNPP compared to the interaction between precipitation and nematode presence.

fBNPP	Df	F value	Pr(>F)
Soil Moisture	1	1.4636	0.2327
Nematode	1	0.0493	0.8253
Soil Moisture x Nematode	1	1.2214	0.2750
Residuals	45		

Table 6. ANOVA table for a model of fBNPP compared to the interaction between soil moisture and nematode presence.

DISCUSSION

Effects of Precipitation

Precipitation manipulations had mixed effects on the BNPP. The drought treatments had lower BNPP than the control (Figure 3-4), as expected, though the difference was not significant. This was supported by evidence from the literature showing lower BNPP in drought conditions (Frank 2007). One study in a grassland ecosystem found that the BNPP has varied and unclear responses to changes in precipitation, because short pulses of precipitation do not increase soil moisture for long enough to increase plant growth (Byrne et al. 2013). This agrees with the idea that mean annual precipitation and BNPP are usually positively correlated (Hui and Jackson 2006). Arid grasslands specifically may not have strong or large responses to changes in precipitation (Wilcox et al. 2015). Therefore, the BNPP in arid grasslands does not always respond in an expected way to changes in precipitation and soil moisture, which explains the lack of a significant pattern in the BNPP in this study.

The ANPP increased as precipitation and soil moisture increased, though the trend was not significant in either case (Figure 5-6). The trend of increasing ANPP with precipitation in the microcosms fit within the expected pattern for ANPP in grasslands, where greater yearly precipitation often leads to higher ANPP (Sala et al. 2012). Increases in precipitation compared to the mean typically increase the ANPP in grassland ecosystems, though precipitation legacies from previous years can influence the current year ANPP (Reichmann et al. 2013, Wilcox et al. 2015). Legacies can alter the expected ANPP by 20%, which may, in part, explain the low values for ANPP in the microcosms (Reichmann et al. 2013). Greater mean annual precipitation between grasslands also increases aboveground biomass and NPP (Sala et al. 2012, Kang et al. 2013). The ANPP in the microcosms increased as expected, though the effect was not large enough to be significant.

The fBNPP decreased with greater precipitation and soil moisture, though the trend was also not significant (Figure 7-8). The expected decrease in fBNPP resulted from a greater increase in ANPP than the increase in BNPP as precipitation increased. The fBNPP and ratio of belowground to aboveground biomass have been shown to decrease with increasing mean annual precipitation, a pattern which is likely replicated with increased precipitation in one ecosystem (Hui and Jackson 2006, Kang et al. 2013). This pattern may result from changes to plant biomass allocation (Kang et al. 2013). The time of year in which precipitation is received can influence the BNPP more than ANPP, so a drought during part of the growing season can affect the fBNPP (Frank 2007). This precipitation pulse size and timing can influence the response of plants (Schwinning and Sala 2004).

The overall NPP for the microcosms was much lower than NPP values for other studies of NPP at the Jornada (Havstad et al. 2006, Hui and Jackson 2006). The mean ANPP for all the microcosms was 12.9 g/m^2/yr, while mean ANPP values in other studies at the Jornada can range from 81.62 g/m^2/yr to 248 g/m^2/yr (Havstad et al. 2006, Hui and Jackson 2006). The BNPP for the microcosms was also lower than what would be expected from other studies, with a mean BNPP in the microcosms to 20 cm depth of 33.8 g/m^2/yr compared to 228.08 g/m^2/yr from other studies at the Jornada, though this study measured to 30 cm depth. (Hui and Jackson 2006). Though precipitation during the growing season was higher than average, the total biomass and NPP in the microcosms was quite low. One factor influencing observed NPP values may be that all biomass in the microcosms was grown from seed, rather than measured as additional growth from established plants (Sala et al. 2012, Kang et al. 2013). NPP in the microcosms was much lower than what would be expected based on other studies in Chihuahuan Desert grasslands.

Effects of Nematode Inoculation

There were no predator nematodes and very few omnivores in the microcosms, so only lower trophic levels, such as plant parasites, could affect the NPP in the microcosms. This trophic structure was unexpected, as there are predator nematodes in the Chihuahuan Desert, though in relatively small numbers compared to other trophic levels (Vandegehuchte et al. 2015). There were fewer nematodes overall in the defaunated nematode treatment, but the reduction in nematode community size was not significant compared to the inoculated treatment; nematode populations were the same in both nematode treatments. Total nematode numbers in the microcosms at the end of this study were much lower than nematode numbers in other observational studies in the Chihuahuan Desert, a consequence of manipulation of nematode numbers in both nematode treatments; the inoculated treatment may have lost nematodes throughout the season because of the manipulation of soil and biomass (Freckman et al. 1987, Vandegehuchte et al. 2015). Nematode numbers at the end of the study averaged 774 individuals per kilogram of dry soil, compared to a mean of 11,954 individuals per kilogram of dry soil recorded at the Jornada in an observational study previously (Vandegehuchte et al. 2015). The reason behind the low nematode numbers may be a positive correlation between nematode population size and NPP (Yeates 1979). The microcosms had both low nematode numbers and low NPP, indicating that the small plant sizes may have resulted in little biomass for the herbivorous nematodes to eat and thus low nematode numbers.

The nematode treatments had no effect on BNPP (Figure 10). I expected the inoculated treatment to have lower BNPP because of nematode herbivory that would reduce total root biomass (Khan and Kim 2005). Herbivorous nematodes, however, can increase belowground biomass and NPP if plants allocate more energy and growth to their roots to compensate for the belowground herbivory (Bardgett et al. 1999). The nematode treatments also had similar numbers of nematodes (Figure 9). The reason behind the absence of an effect of nematode treatment was most likely this similarity in low nematode numbers between the treatments, despite evidence of different effects of nematodes on BNPP.

In a similar pattern to the BNPP, the ANPP had no pattern in comparison to nematode treatment (Figure 11). I expected nematode inoculation to decrease the ANPP, with herbivory negatively affecting aboveground plant growth as well as belowground growth (Figure 1). Some plants can successfully maintain aboveground growth even with belowground herbivory (Mundim et al. 2017). Aboveground growth rate can remain stable despite nematode herbivory, because plants can direct energy to protecting leaves while still maintaining root growth (Mundim et al. 2017). This is supported by the fact that there were no predator nematodes in the microcosms; the whole effect of nematodes on NPP was from the lower trophic levels and from herbivory. The effect of herbivory, however, was also likely not observed, because there was no difference in the nematode numbers between treatments.

The fBNPP did not change between the nematode treatments, likely a result of the absence of any effect on the BNPP and the ANPP (Figure 12). Mundim et al. (2017) showed that nematode herbivory has little effect on the allocation of growth in plants, only slightly increasing root growth. This may explain why there was no difference in fBNPP between the nematode treatments. Another factor contributing to the lack of an effect of nematode inoculation is the low numbers of total nematodes in both of the treatments.

Effects of the Interaction between Precipitation and Nematode Inoculation

The presence of nematodes increased the BNPP in drought treatments and decreased it in irrigated treatments, though the difference was not significant (Figure 3-4; Tables 1-2). There was no effect of nematodes across the whole range of precipitation treatments. Nematode trophic levels in the Chihuahuan Desert are not affected by precipitation, and have responses to changes in soil moisture that are not consistent with their trophic level and vary by year (Vandegehuchte et al. 2015). Increases in total precipitation do not affect the total number of nematodes in desert soils, which means that irrigation would have limited effect on the total numbers of nematodes (Freckman et al. 1987). Nematode numbers were the same between nematode treatments, which eliminated the effect of an interaction with precipitation or soil moisture. No differences in nematode numbers occurred between nematode treatments, and consequently the response of BNPP to the interaction between precipitation and nematode treatment was not consistent.

There was no overall pattern to the interaction between precipitation or soil moisture and nematode presence for ANPP (Figures 5-6; Tables 3-4). This can be explained both by the similarity of nematode numbers in both treatments and the effect of nematodes on ANPP. Chihuahuan Desert nematode populations are not affected by changes in soil moisture, suggesting that the ANPP would respond in the same way in all precipitation treatments (Vandegehuchte et al. 2015). Likewise, nematode herbivory has little effect on aboveground biomass, indicating that plants protect leaves when they experience nematode herbivory (Mundim et al. 2017). The effect of the interaction of nematode presence and precipitation was not consistent, indicating that the similarity of nematode numbers between nematode treatments erased any interaction effect between nematodes and precipitation.

The fBNPP showed no overall pattern for the interaction of precipitation or soil moisture and nematode presence, with no significant trends (Figures 7-8; Tables 5-6). The fBNPP is influenced by the trends in both ANPP and BNPP. Precipitation was expected to affect this relationship, but the results demonstrate that this was not the case. In general, nematode herbivory has a neutral effect on the partitioning of plant growth, because neither aboveground nor belowground biomass are significantly affected (Mundim et al. 2017). The similarity of the fBNPP between different precipitation treatments could be influenced by the nematode community; the desert nematode community does not respond to changes in precipitation (Vandegehuchte et al. 2015). This could also result in the similarity of the fBNPP between nematode treatments in the different precipitation treatments. Another factor influencing the lack of a pattern in the fBNPP may be the lack of a trend in the ANPP compared to the interaction of precipitation and nematode presence (Figure 5). This would make the fBNPP vary with no pattern throughout the precipitation and nematode treatments.

Conclusion

The BNPP, ANPP, and fBNPP did not change in any significant way with precipitation and nematode treatments, though some trends in the data supported the hypotheses. This suggests that nematodes and precipitation may both influence the division of plant growth. One factor that affected the results in this study is the lack of an effect of the nematode inoculation at the end of the season, with much lower nematode densities in the microcosms as compared to other studies of nematodes in the Chihuahuan Desert (Figure 9). The difficulty in maintaining live seedlings may have resulted in low nematode populations in addition to low NPP values. The unclear results for the BNPP, ANPP, and fBNPP in relation to nematode trophic levels in this experiment suggest that further study is needed to understand these components of NPP and what factors control them.

This experiment had several limitations that I would fix if I were to repeat this experiment. The experiment should be replicated over several years to ensure that the conditions of one particular year do not affect the results; for example, a rainy year or a dry year could influence plant growth even in a control treatment. The seedlings used for the study should be grown earlier in the year, so that the average plant size is larger by the time the seedlings are put in the field at the beginning of the growing season. The overall nematode numbers in the inoculated treatment should also be monitored throughout the season to ensure that a difference between the two nematode treatments remains and that nematode populations are not decreasing in the inoculation treatment. Finally, effects of dust in the field on nematode numbers in the defaunated treatment should be recorded, to learn whether or not there are significant numbers of nematodes introduced to the defaunated treatment in this way.

This study demonstrates that there are potential trends in NPP in relation to precipitation. Though no relationship with nematode presence was observed, the limitations of the study may have influenced the lack of a trend. Future research on this topic should address the shortcomings of this experiment and address some of the factors that may have limited the effectiveness of the experiment.

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