The effect of nutrient and water availability on grasshopper herbivory

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Abstract

Nutrient and water supply are important for healthy ecosystems. They influence plant growth as well as animal behavior. This study looks at how nutrient and water availability affects the feeding habits of a grass specialist grasshopper, *Ageneotettix deorum*. The field study took place in a grassland site dominated by *Pseudoroegneria spicata* on the National Bison Range in Montana, USA. Cages either had grasshoppers inside of them or not and were treated with nitrogen, water, water pillows, nitrogen +water, water +water pillows, nitrogen + water pillows, nitrogen +water pillows + water, or a control. Remaining biomass at the end of the three week field study were compared across treatments. In the lab, grasshopper preference for control grass, water treated grass, nitrogen treated grass, and nitrogen +water treated grass was tested. Analysis of the field data revealed that cages treated with both nitrogen and some form of water had more grass remaining than cages without both of those treatments ($p=0.064$). When water weight was added back to the dried mass, the nitrogen + water treated grass had more remaining than the nitrogen treated, water treated, and control grasses ($p<0.01$). In the lab, the control and nitrogen + water treated grasses were eaten more than the nitrogen treated and control treated grasses ($p<0.001$). These results could suggest that added nitrogen and water in the grass could influence how much grasshoppers eat, which could have impacts on the rate of nutrient cycling. Altered nutrient cycling could have wide reaching impacts on grassland health.

Introduction

Understanding how environmental factors influence animal behavior is essential for determining how ecosystems will change in the future given the predicted impacts of climate change (McCluney and Sabo 2009). Dry regions are expected to be especially vulnerable to global climate change and desertification (McCluney et al. 2012), making them even drier. Given this possibility, it is important to consider how water availability affects the behavior of animals in dry regions. Freshwater is essential to terrestrial life, but it is in low supply on the surface of the Earth, since 99% of all freshwater is located in underground aquifers (Winter et al. 1998Of course, water is not the only important resource for animals to consume. They also need nutrients, such as nitrogen, which is often limiting. The nutrient content of plants has been recognized for years as a critical component that influences herbivore success (Fagan et al. 2002). Grasshoppers are the dominant invertebrate herbivore in grassland ecosystems (Zhang et al. 2011) and have been noted to significantly increase nutrient cycling and plant production (Belovsky and Slade 2000; Belovsky 2000).

This study sets out to examine how grasshopper herbivory is affected by the availability of nitrogen and water in and around grass. The grasshopper species that will be used in my experiments is *Ageneotettix deorum*, which is a slantfaced grasshopper that feeds primarily on grasses. It has a wide range (Figure 1), its preferred habitat is mixedgrass or bunchgrass prairies, and it is considered to be a pest species in many rangelands (Pfadt 1994). I stocked grasshoppers into cages with high or low soil moisture and then added nitrogen (fertilizer), water pillows, water pillows and nitrogen, or nothing as a control. I hypothesized that grasshoppers would eat control grass the most, because it should be the least nutrient and water rich. I expect grass treated with nitrogen to be eaten less than grass not treated with nitrogen since it should be nutrient rich and a grasshopper should not need to eat as much. I expect grass treated with water to be eaten less than controls but more than nitrogen treated grass because more water should increase digestion rates. I expect cages treated with nitrogen and water to have the least amount of grass eaten, since it should be high in nutrients and water and the grasshoppers should not need to eat as much.

I also conducted laboratory feeding trials. I hypothesized that the nitrogen + water treated grass would be eaten the most, followed by the water treated grass, then the nutrient treated grass, and the control grass being eaten the least, because of the aforementioned relative nutritional properties of the grasses.

Methods

Field Study

In order to document the effects of nutrient and water availability on grasshopper herbivory, 64 cages were set up in an ungrazed, *Pseudoroegneria spicata* dominated site on the National Bison Range in Montana, USA. The cages were 0.1m^2 in basal area and constructed of aluminum screening (see Belovsky and Slade 1995 for full description of cages). The 64 cages were split into two groups: ones that were watered regularly, and ones that were not watered. Within each of these, 8 were fertilized, 8 had a water pillow placed inside, 8 were fertilized and had a water pillow inside, and 8 had no additional treatment. In each of these subgroups, 4 cages had 3 grasshoppers placed inside, and the other 4 served as controls for that specific treatment.

The grasshoppers in the cages were *A. deorum*, a grass feeding specialist. Grasshoppers in each cage were censused every 3 days, and missing grassshoppers were replaced as needed. Every three days, after grasshoppers were censused, dried up water pillows were replaced with wet ones, and 0.5 liters of water were added to the necessary cages using a watering can. The fertilized cages were fertilized once at the beginning of the experiment with 0.7g of fertilizer (Miracle Gro All Purpose Plant Food) added to each of cage in the fertilized treatments (7g N/m^2). .

The experiment started in the morning of July 12th, 2013 and ended in the morning of August 1st, 2013. At the end of the experiment, cages were removed and the standing grass and the grass litter from each cage was clipped, dried at 60°C for 72 hours and weighed.

Statistical Analysis

Litter- To determine if the treatments had effects on the amount of litter, the litter biomass in cages without grasshoppers was compared across treatments using a one-way Analysis of Variance (ANOVA). A Kruskal-Wallis test was used to determine whether the amount of litter left in cages with grasshoppers varied among treatments. A one-way ANOVA was used to test for differences between the treatments with and without grasshoppers.

<u>Grasses-</u> I used two one-way ANOVA's to test for differences in grass biomass among treatment in cages with and without grasshoppers. To make comparisons among treatments, I

pooled treatments that were treated with nitrogen and compared them to all the treatments that had no nitrogen. I also compared the pooled nitrogen treatments to just the controls. I compared the nitrogen pooled data to non-nitrogen treatments and the controls using one way ANOVA's. I followed that same procedure with water treated cages and cages with water pillows. The remaining biomass of grass in grasshopper cages that were treated with nitrogen and water (in either form) were combined and compared to the remaining biomass of the other grasses using a one way ANOVA. The mass data were first transformed using a log transformation for this test in order to normalize it and meet the assumptions of an ANOVA. Percent water loss (calculated from the lab study below) was added back to the mass of the control, nitrogen, water, and nitrogen + water treated grasses. The difference between these treatments was then determined using a one-way ANOVA with a Tukey's Post Hoc Test to determine which specific treatments were different from one another.

Lab Study

Grasshopper feeding preference for grasses from each treatment (watered, nitrogen added, watered and nitrogen added, control) was tested by placing a grasshopper in a mason jar with three bunches of treated grasses and one bunch of control grass. The grass for these lab trials was treated for two weeks prior to the feeding trials. 0.3g of each treatment grass was placed into the jars. The grasshoppers were starved for 7 hours prior to the trials. There were 10 jars with one grasshopper inside, half of them with females and half of them with males. There were also 3 jars with 0.2g of each treatment grass but without any grasshoppers to account for the percent water loss over the course of the trials. The trials lasted for 6 hours and each bunch of grass was re-weighed afterwards to determine how much was consumed by the grasshoppers.

The percent water loss for each treatment of grass was determined from the grasses that were in the jars without any grasshoppers by measuring the difference between the initial and final weights and then dividing that by the initial weight and multiplying by one hundred. This amount of water loss and the final weight was then subtracted from the initial weight of grass to find the amount of grass that was eaten. Any negative values were made into zeroes.

Statistical Analysis

To compare the amount eaten for each treatment, as Kruskal-Wallis was used since the data could not be normalized. A series of Mann-Whitney U tests were used to conduct pair-wise comparisons among treatments. A Kruskal-Wallis was used to see if there was a difference in the amount eaten between the genders. A Kruskal-Wallis was also used to determine if there were differences in the amount females ate between the treatments. A series of Mann-Whitney U tests were used to find what the exact differences were. The same statistical procedure was used to analyze the male data.

All statistical tests were done in R. All data transformations and graphs were done in Excel. P values of 0.1 or smaller were interpreted as significant.

Results

Litter

There were no differences in the biomass of litter among treatments in cages without grasshoppers (F=0.844, df=7, p=0.562). There were also no differences in litter biomass among treatments in cages withgrasshoppers (df=7, p=0.7084). There was no difference in litter biomass remaining between the grasshopper and non-grasshopper cages (F=0.509, df=15, p=0.923).

Standing Grass

Treatments had no effect on grass biomass in the cages without grasshoppers ($F=1.078$, $df=7$, $p=0.407$). There were no differences in remaining grass biomass among treatments in cages with grasshoppers ($F=1.532$, $df=7$, $p=0.204$). When grouped, however, the cages that had been treated with both nitrogen and some form of water had significantly more remaining grass biomass than the cages without both nitrogen and water treatments ($F=3.685$, $df=1$, $p=0.064$) (Figure 2). When percent water was added back to the remaining grass biomass in the grasshopper cages, there was significantly more ($F=6.33$, $df=3$, $p<0.01$) (Figure 3) in the cage treated with nitrogen + water than in the control cage ($p=0.0109$), and the cages treated by nitrogen ($p=0.0279$) and water ($p=0.0217$) only. When grouped together, cages that were treated with nitrogen, water, and water pillows did not have significantly different amounts of remaining grass biomass than the control cages ($F=2.362$, $df=1$, $p=0.147$; $F=1.394$, $df=1$, $p=0.253$; $F=2.652$, $df=1$, $p=0.121$).

Feeding preference

Male *A. deorum* ate more than their female counterparts (Kruskal-Wallis chi-squared=4.3942, $df=1$, $p=0.036$) (Figure 4). Treatment affected how much was eaten for both males (Kruskal-Wallis chi-squared=14.3827, $df=3$, $p<0.01$) (Figure 5) and females (Kruskal-Wallis chi-squared=9.6534, $df=3$, $p=0.022$) (Figure 6). Females consumed control grass and nitrogen + water treated grasses more than the nitrogen treated ($W=22.5$, $p=0.025$; $W=5$, $p=0.072$) and water treated grasses ($W=22.5$, $p=0.025$; $W=20$, $p=0.072$). Males also consumed the control grass and nitrogen + water treated grasses more than the nitrogen treated ($W=21$, $p=0.09$; $W=0$, $p=0.012$) and the water treated ($W=22$, $p=0.045$; $W=25$, $p<0.01$) grasses.

When males and females were combined together, there were differences in the amounts eaten of the control, nitrogen, water, and nitrogen + water treated grasses (Kruskal-Wallis chi-squared=20.1492, df=3, $p<0.001$) (Figure 7). More of the control was eaten than the nitrogen treated (W=86, $p<0.01$) and water treated (W=89, $p<0.01$) grasses. More of the nitrogen + water treated grass was eaten than the nitrogen treated (W=13, $p<0.01$) and water treated (W=89, $p<0.01$) grasses.

Discussion

This experiment studied grasshopper responses to fertilization and water in field enclosures. I found that the litter biomass and standing grass biomass at the end of the experiment did not differ among treatments. There were also not immediately obvious differences in remaining biomass in the grasshopper cages between treatments. However, by observing general trends in remaining grass biomass, there was less grass eaten in cages treated with nitrogen and water than in other treatments.

The fact that the only differences in the remaining grass biomass in grasshopper cages occurred when all treatments involving nitrogen and some form of water were grouped together and compared to those without both nitrogen and water is important. The added nitrogen should have made the grass more palatable and nutrient rich, meaning that the grasshoppers should need to eat less to get the amount of nutrients they need. Since grasshopper densities inside the cages were held constant across treatments throughout the experiment, it makes sense that this treatment had the most grass remaining. Had grasshopper populations been allowed to die off, it is likely that this treatment may have had the least amount of grass remaining since grasshoppers probably would have survived the best under conditions with the most nutrients.

If this were the whole story, then the cages treated with just nitrogen should have also seen a smaller amount of grass eaten, but this was not the case. This could be because without added water to the cages, the grass may not have actually been able to take up and use the added nitrogen since the summer was very dry and the soil moisture at the study site was exceptionally low. If this is the case, then it may still not make sense for the nitrogen and water pillow treatment to have been grouped with the other nitrogen and water treatments. However, the water pillows could have increased humidity in the cages, which would have allowed for stomata in the grass to stay open longer, increasing photosynthesis and leading to higher quality food resource for grasshoppers. If this is the case, then grasshoppers would need to eat less. Water pillows also provide more water for the grasshoppers to drink, which is especially important in dry regions, such as my research site in the National Bison Range (NBR), where water scarce. The NBR only gets around 35cm/yr of precipitation a year (Belovsky 2000), making what water is present all the more precious to herbivorous insects that must forage for water and other nutrients. If they have more water available, they should presumably need to eat less grass. Another reason that the nitrogen + water treated grasses were eaten less could be because the added nitrogen could have allowed the grass itself to invest more nutrients into chemical or structural defenses, making the grasshoppers less likely to eat it.

The fact that litter amount in both grasshopper and non-grasshopper cages had no differences between treatments and that the treatments had no impact on the growth of grass says something interesting about the site where the field study was set up. The treatments having no impact on the biomass growth is surprising, but it could be this way because the treatments were added too late into the summer, and what really determined the amount of grass in each cage were the site’s historical conditions over the past years. This idea is supported by the similar trends observed in amounts of standing grass and grass litter in the cages without any grasshoppers.

In the lab study, grasshoppers consumed more of the nitrogen + water treated grass than either the water treated or nitrogen treated grasses. This is not surprising, because the nitrogen + water treatment should be more nutrient and water rich than the nitrogen or water treatments. However, grasshoppers also consumed more of the control grasses compared to the the water treated and nitrogen treated grasses. It is unclear why this happened. All three of the other treatments should have the grass more palatable by increasing water and nitrogen content. It's possible that all of the treatments somehow increased the amount of defensive chemicals the grass produced, therein making the grass less palatable and that the nitrogen + water treated grass had such high quality nutrients and water that the grasshoppers still preferred it, despite the defensive chemicals. Furthermore, it is possible that the abundance of some micronutrients in the control grass was higher than in the others, and the grasshoppers ate the control grass more in order to access those nutrients. A past study has shown that grasshopper density has been influenced by the presence of micronutrients in the grass (Joern et al. 2012).

It was also interesting to note that in the lab study, the male grasshoppers ate more than their female counterparts, given that females tend to be larger. It is possible that females have more fat reserves so that during the starving process, they were less stressed than the males, so when they were presented with grass they did not need to eat as much.

These results are important given current agricultural trends and future climatic shifts. Human alterations of the nitrogen cycle through fossil fuel combustion and production of nitrogen fertilizers increase N availability (Vitousek et al. 1997) in ecosystems, making it important to know how increased nitrogen affects grasshopper herbivory. The decreased amount of herbivory on the grass treated with nitrogen and water could have implications for nutrient cycling, which could have wide ranging impacts on grassland health and diversity.

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Literature Cited

Belovsky, G. E. 2000. Do Grasshoppers Diminish Grassland Productivity? Grasshopper and Grassland Health 7-29.

Belovsky, G. E., and J. B. Slade. 2000. Insect Herbivory Accelerates Nutrient Cycling and Increases Plant Production. National Academy of Sciences 97: 14412-14417.

Fagan, W. F., E. Siemann, C. Mitter, R. F. Denno, A. F. Huberty, H. A. Woods, and J. J. Elser. 2002. Nitrogen in Insects: Implications for Trophic Complexity and Species Diversification. The American Naturalist 106: 784-802.

Joern, A., T. Provin, and S. T. Behmer. 2012. Not just the usual suspects: Insect herbivore populations and communities are associated with multiple plant nutrients. Ecology 93(5): 1002-1015.

McCluney, K. E., J. Belnap, S. L. Collins, A. L. González, E. M. Hagen, J. N. Holland, B. P. Kotler, F. T. Maestre, S. D. Smith, and B. O. Wolf. 2012. Shifting species interactions in terrestrial dryland ecosystems under altered water availability and climate change. Biological Reviews 87: 563-582.

McCluney, K. E., and J. L. Sabo. 2009. Water availability directly determines per capita consumption at two trophic levels. Ecology 90: 1463-1469.

Pfadt, R. E. 1994. Field Guide to Common Grasshoppers. University of Wyoming.

Redak, R. A., and J. L. Capinera. 1994. Changes in western wheatgrass foliage quality following defolation: consequences for a graminivorous grasshopper. Oeecologia 100: 80-88.

Reynolds, J. F., D. M. S. Smith, E. F. Lambin, B. L. Turner II, M. Mortimore, S. P. J. Batterbury, T. E. Downing, H. Dowlatabadi, R. J Fernández, J. E. Herrick, E. Huber-Sannwald, H.

Jiang, R. Leemans, T. Lynam, F. T. Maestre, M. Ayzara, and B. Walker. 2007. Global Desertification: Building a Science for Dryland Development. Science 316: 847-851.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human Alteration of the Global Nitrogen Cycle: Sources and Consequences. Ecological Applications 7(3): 737-750.

Winter, T. C., J. W. Harvey, O. L. Franke, and W. M. Alley. 1998. Ground water and surface water: a single resource.*in* U. S. G. Survey, editor. Circular 1139, Reston, VA, USA.

Zhang, G., X. Han, and J. J. Elser. 2011. Rapid top-down regulation of plant C:N:P stoichiometry by grasshoppers in an Inner Mongolia grassland ecosystem. Oecologia 166: 253-264.

Figures

Figure 1: The geographic range of *Ageneotettix deorum* in the continental United States (Pfadt 1994).

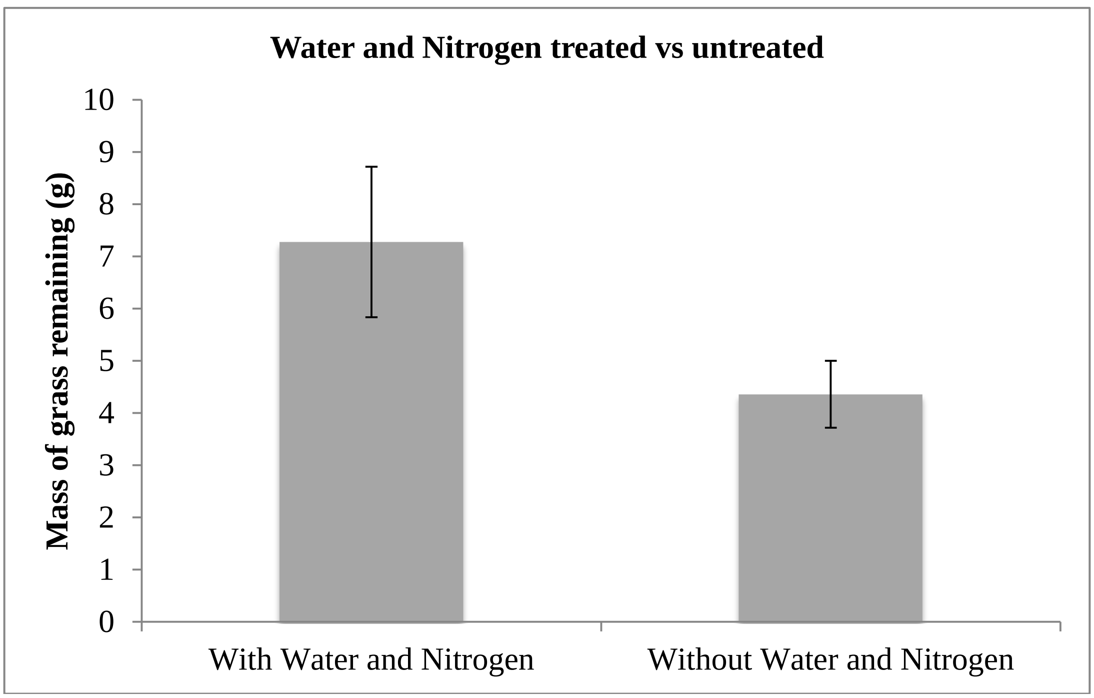

Figure 2: Remaining grass in cages treated with nitrogen and some form of water compared to all other treatments. There was more grass remaining when grasses were treated with both nitrogen and some form of water (p=0.064).

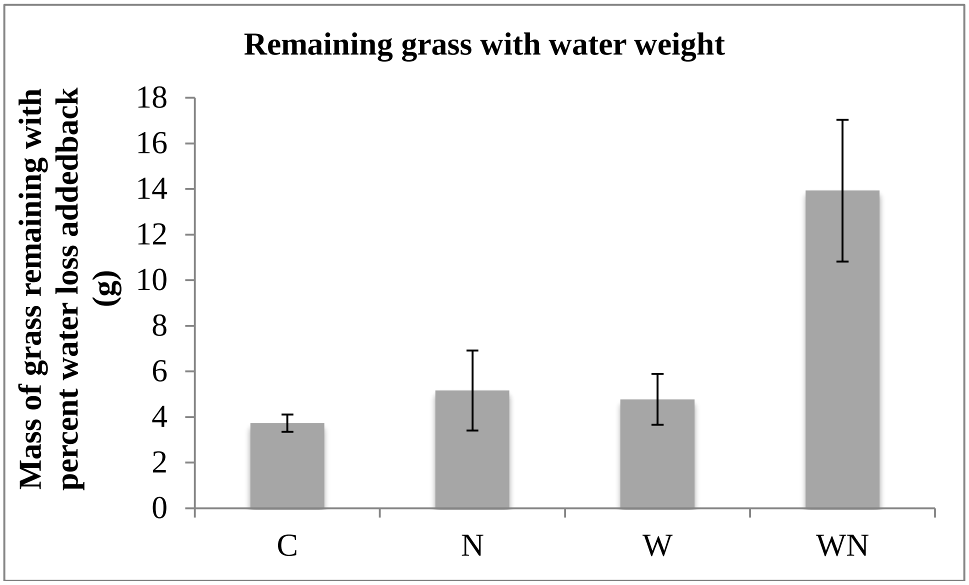

Figure 3: Remaining grass biomass when water loss is added back in. Treatment had a significant impact on the remaining biomass ($p<0.01$). The nitrogen + water treatment had more remaining than the control, nitrogen treated, and water treated grasses (SE=10.2, $p=0.01$; SE=8.8, $p=0.02$; SE=9.2. $p=0.02$).

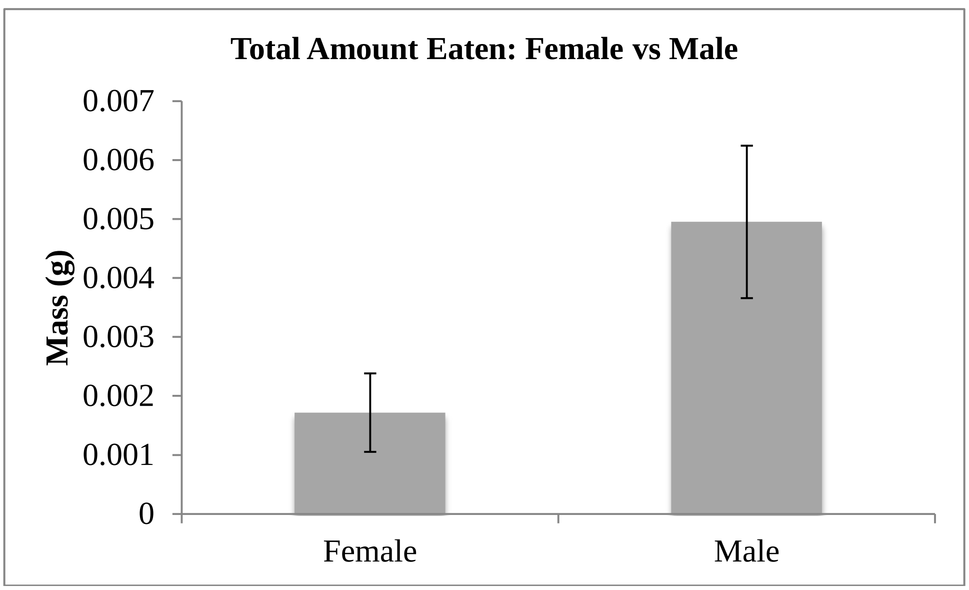

Figure 4: Difference in amount eaten between *A. deorum* males and females. Males ate more than their female counterparts ($p=0.036$).

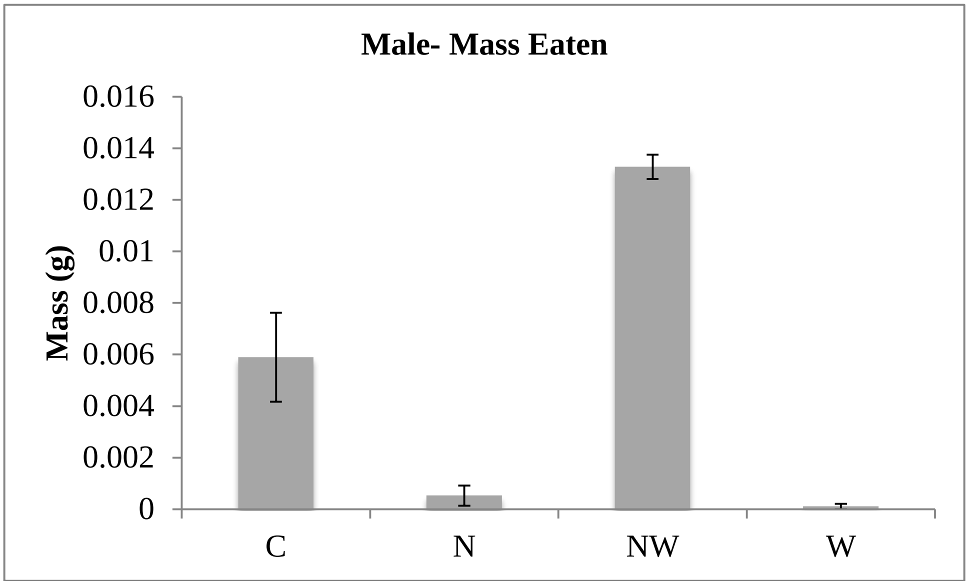

Figure 5: Treatment effects on the amount male *A. deorum* ate. Treatment had a significant impact on the amount of grass the males ate ($p<0.01$). They ate more nitrogen + water treated grass than the water treated, the nitrogen treated, and the control grasses ($p<0.01$; $p=0.01$; $p=0.04$).

Figure 6: Treatment effects on the amount female *A. deorum* ate. Treatment had a significant impact on the amount of grass that females ate ($p=0.02$). The control grass was eaten more than the nitrogen treated and water treated grasses ($p=0.02$; $p=0.02$). The nitrogen + water treated grass was eaten more than the nitrogen treated and water treated grasses ($p=0.07$; $p=0.07$).

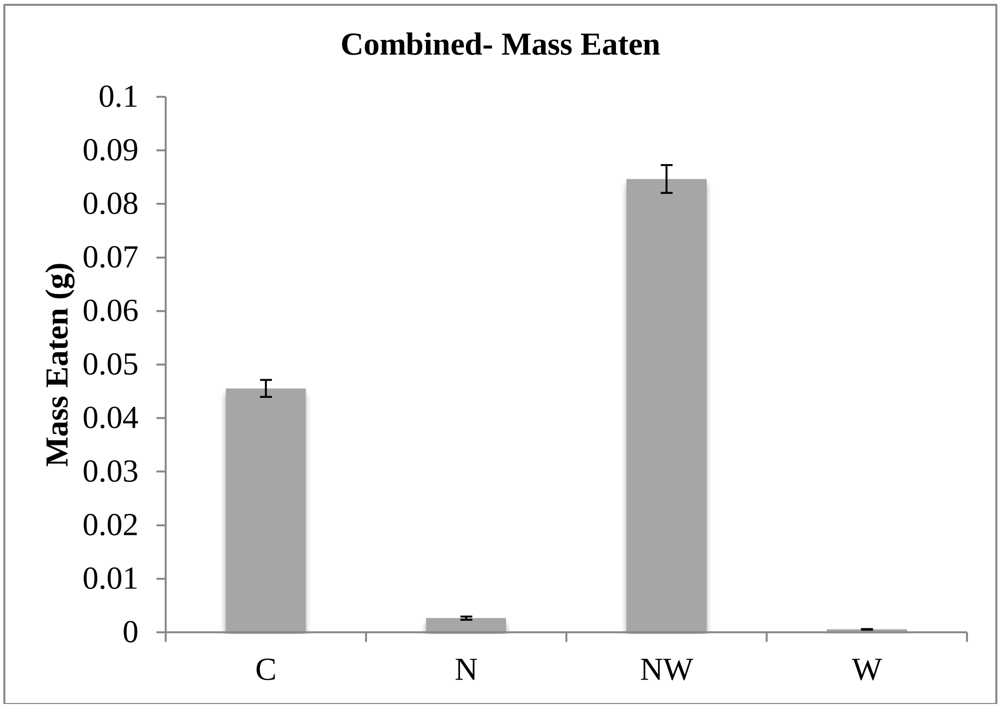

Figure 7: Treatment effects on both male and female *A. deorum*. Treatment had a significant effect on the amount of grass the grasshoppers ate ($p<0.001$). The control grass was eaten more than the nitrogen treated and water treated grasses ($p<0.01$; $p<0.01$). The nitrogen + water treated grass was eaten more than the nitrogen treated and water treated grasses ($p<0.01$; $p<0.01$).