

Title: Effects of simulated cattle hoof depressions on animal diversity and abundance in a mountain bog

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Abstract

Cattle have a long history in the Appalachian mountains and privately owned land may be subjected to the effects of cattle grazing. This is especially true of mountain bogs, most of which occur on private land that has been converted to cattle pasture. Cattle may have devastating effects on plants, as they graze indiscriminately. The effects of cattle walking through a mountain bog, however, may also be beneficial to animals that require water. This may be the case because as cattle walk through bogs, their hooves create water-filled depressions that may create refugia for animals. No prior studies have looked at the effect that cattle hoof depressions have on the biodiversity and ecological community of mountain bogs. My study aims to determine if these depressions are used as refugia by using an auger to create simulated cattle hoof prints and collecting data on animals found in these depressions. This study consisted of monitoring plots with auger holes and plots without auger holes. The number of animals in each plot was observed over the course of 12 weeks. The results of my study show that plots with auger holes have significantly greater taxon richness and animal abundance. These results indicate that some taxa, especially invertebrates, may be attracted to these water-filled hoof depressions.

1. Introduction

Mountain bogs and fens are rare habitats in the Southern Appalachian Mountains¹. These habitats, which I will refer to collectively as bogs, form in poorly drained depressions in the mountains. This is different from Northern Bogs which form following glacial recession². This type of habitat is very rare mainly due to habitat loss via the intentional draining of bogs for development³. Climate change has also had a negative impact on bog ecosystems⁴. Mountain bogs support high biodiversity including many rare and endemic species such as the green pitcher plant (*Sarracenia oreophila*), mountain sweet pitcher plant (*Sarracenia jonesii*), bunched arrowhead (*Sagittaria fasciculata*), and the bog turtle (*Glyptemys muhlenbergii*)⁵. Each of these species is federally listed and is only found in bogs⁶. Further, 90% of all rare species in North Carolina occur in bogs⁷.

In addition to habitat loss, many bogs are also exposed to the effects of cattle grazing⁸. Many of the mountain bogs in North Carolina are found on private land and have been converted to cattle pasture⁹. Cattle not only graze on and crush fragile plants that occur in the bogs, but may also compress the soil, which could be harmful to burrowing animals¹⁰. They also create holes in the mud where they walk. These holes can fill with water and might provide refugia for animals when the bog begins to dry. These water-filled depressions may help maintain biodiversity in

bogs. Due to the rarity of this type of habitat and the many endemic species that reside here, understanding how cattle impact the ecology of mountain bogs and Southern Appalachian fens is crucial for conservation work.

Although prior studies have speculated that grazing impacts mountain bog habitats, none have looked at how the depressions that cattle create while walking through bogs affect the biodiversity of bogs. One study that took place in New York¹¹ found that livestock grazing was actually good for bog turtle populations because it helped to keep vegetation from encroaching on wetlands, which is the bog turtles' primary habitat. The authors proposed using grazing animals as a way to promote the conservation of northern bogs¹². The hypothesis that the presence of cattle may actually benefit bog turtles is also supported by a study done in New Jersey that examined whether cattle grazing could be used as a way to help conserve the habitat on which bog turtles rely¹³. The authors found that bog turtles were more abundant in areas with cattle than in areas without cattle, although the exact reasons for this remain unclear. They also found that grazing was an effective means of preserving bog habitat. These studies were conducted in northern bogs, which characteristically are very different from a Southern Appalachian Bog. Historically, grazers such as elk may have kept bogs from becoming overgrown in the Southern Appalachians and these large ruminants may have helped maintain suitable habitats for bog turtles¹⁴. In the absence of native grazers, cattle may provide this same service.

Although prior studies have suggested a link between certain bog species such as the bog turtle and the presence of cattle, the mechanism of cattle's effect on biodiversity remains unclear. My study aims to find out how the hoof depressions created by cattle walking through a wetland affect the biodiversity of a bog. For this study, cattle hoof depressions were simulated using an auger. Creating artificial holes allowed me to isolate the effect of hoof depressions, independent of the effects of cattle grazing, and their indirect effects on animal communities.

2. Methods

2.1. Study Site

This study was conducted at a wetland site in Sandy Bottom Preserve (Buncombe County, NC). Wetlands are home to a third of all threatened and endangered species in the USA. They also provide habitat for generalist species¹⁵. Sandy Bottom was an ideal site for this study as it consists of mountain bogs and wetlands. Sandy Bottom has a high biodiversity of plants and animals, especially reptiles and amphibians, due to the many habitats that are present in such a small area. Habitats found at Sandy Bottom include bogs, vernal pools, two permanent ponds, and a stream¹⁶.

2.2. Establishing Plots and Experimental Treatments

I simulated cattle hoof prints in experimental plots within the bog and compared the animal diversity between these and control plots. To establish plots, I used a wetland delineation map generated by the North Carolina Wildlife Resources Commission in 2019. This delineation was then processed to generate sampling plots with the assistance of the US Fish and Wildlife Service, Asheville Field Office. Specifically, ArcGIS Pro (ESRI Inc., Redlands, CA) tool Calculate Fish Net was used to create a grid over the wetland. Each square in the grid was 4m by 4m. Next, the wetland boundary of Sandy Bottom was measured and the grid was trimmed down. The final map consisted of 1256 total squares. Squares were numbered on the grid from 1 to 1256. A random number table was used to select 16 squares from the map to use as plots. Eight control plots and eight experimental plots were alternately assigned (Figure 1). After plots were selected this map was exported as a GeoPdf for use in the Avenza Maps app (Avenza Systems Inc., Toronto, CA). Satellite GPS was used via Avenza to locate quadrants at Sandy Bottom.

In every 4m by 4m quadrant, 1m x 1m plots were set up. Each plot was marked with 4 red flags in the corner. To mimic holes created by cattle hooves, two holes were dug near opposite corners of experimental plots using a handheld auger with a 6.35-centimeter diameter. No holes were created for control plots. Plots were left undisturbed

for a week to allow the flora and fauna to readjust to the new holes. Plants found in plots consisted of alder, blueberry bushes, Japanese stilt grass, sphagnum moss, and other species of grass.

2.3. Data Collection

Starting on July 9th, 2022, 10-minute timed surveys were done at each plot. Surveys consisted of a visual scan for animals present at eye level (tops of plants). I then worked top-down (starting at the tops of plants and going downward) turning over leaves and plants and counting animals. Finally, I scanned the ground for animals. For the experimental plots, a Hillman 48-in Orange Reflective Rod was used to probe the sides of the hole created by the auger to search for animals. Animals found in the auger hole were pulled up for identification and immediately returned to the hole. Animals that were unable to be identified in the field were counted but excluded from further analysis. Plant species occurring at each plot were also identified, but not counted. These data were entered into Excel (Microsoft Corporation, Redmond, WA) and R¹⁷ for analysis using the packages *vegan*¹⁸ and *rcompanion*¹⁹.

2.4. Data analysis

Abundance was calculated by finding the sum of all individuals of each taxon that were identified in control and experimental plots over the course of 12 weeks. Species richness was calculated by determining the number of species occurring in each plot over the course of 12 weeks. Shreiner Ray Hare analysis for nonparametric two-way factorial design was used on the dataset. This analysis was used to evaluate the effects of date, treatment, and their interaction on both species richness and abundance. This test did not account for repeated sampling of the plots over time. Principal component analysis (PCA) was used to evaluate if the community differed between experimental and control plots.

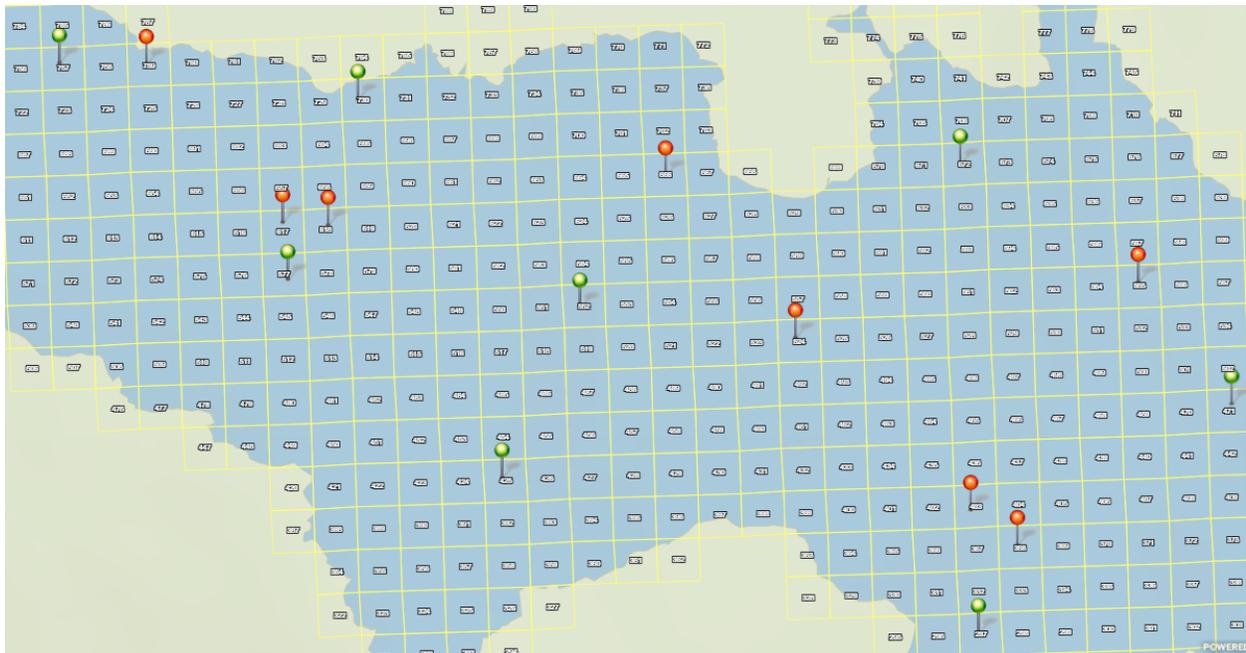


Figure 1. Red pins represent experimental plots (368,617,524,666,403,618,759,565). Green pins represent control plots (672,297,552,757,730,577,471,425).

3. Results

3.1. Animal Abundance

Over the course of 12 weeks, 1194 total animals were found. Of these 1194, 42 organisms were unable to be identified in the field and were listed as “other”. The “other” group was excluded from further analysis. Spiders had the highest overall abundance of all taxonomic groups for both experimental and control plots (Figure 2). The Shreiner Ray Hare analysis showed that date, treatment, and their interaction had significant effects on animal abundance (Table 1, Figures 3 & 4). The total abundance peaked during weeks 8/6/22 and 8/13/22 and was lowest on 22/9/22 (Figure 3). In all weeks, median abundance was equal to or higher in experimental plots than in control plots, and differences between treatments were greater earlier in the season. Out of the 8 taxa, only the abundance of crayfish and ants differed significantly between control and experimental plots (Tukey HSD test, $p < 0.05$).

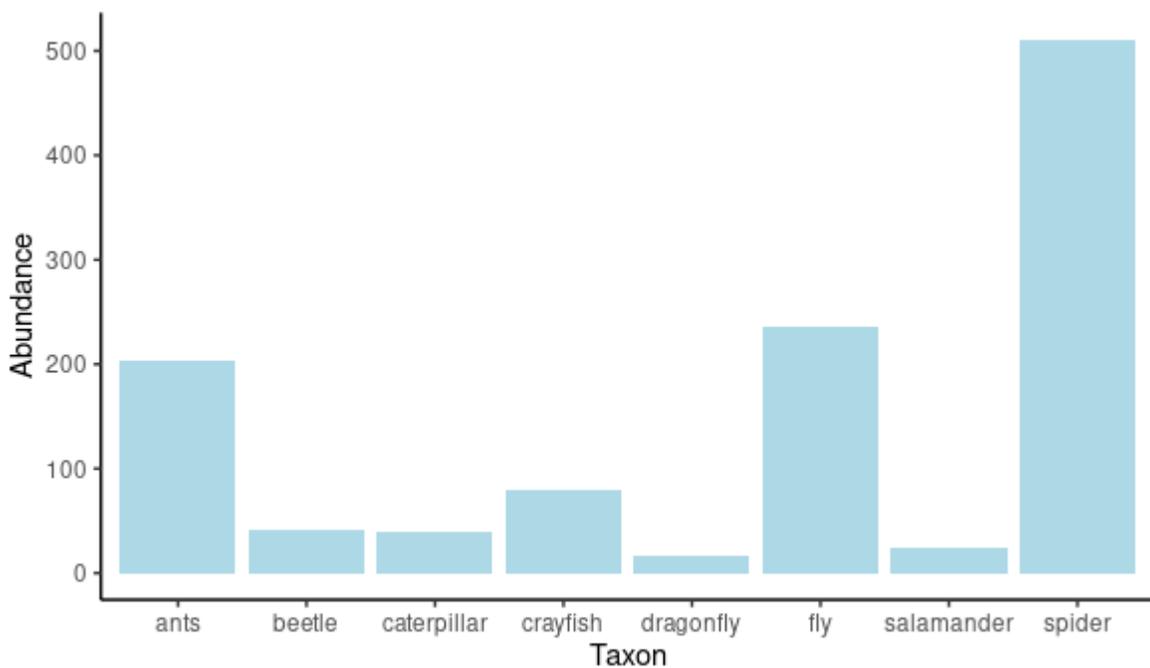


Figure 2. Total abundance for the 8 taxonomic groups of animals surveyed in control and experimental plots.

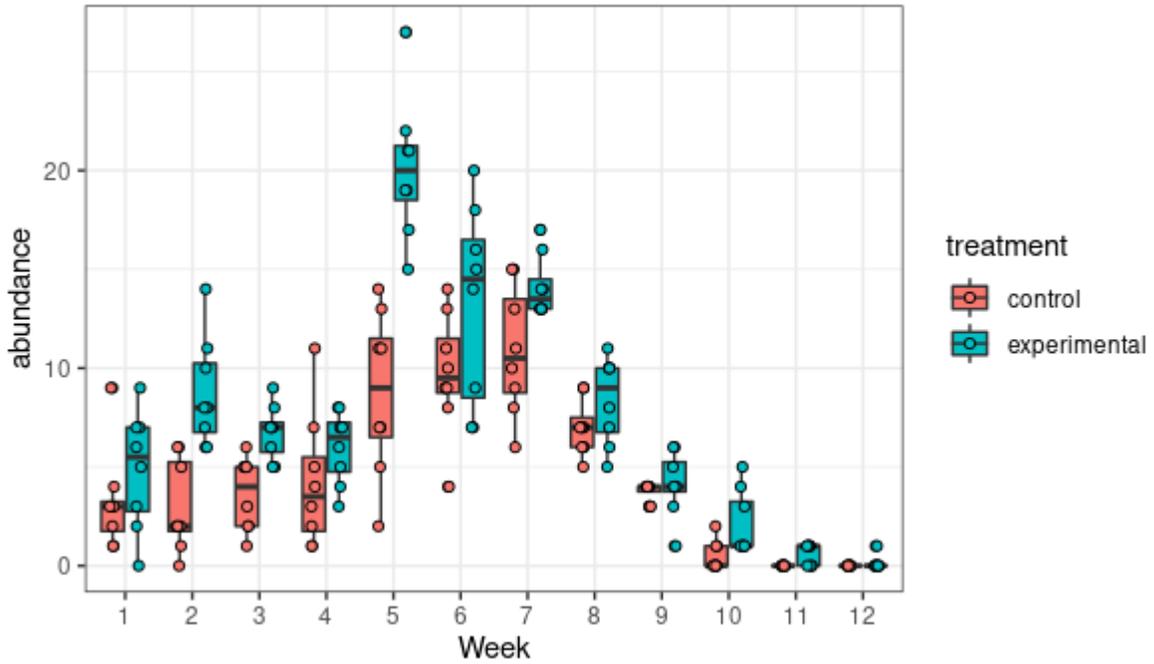


Figure 3. Boxplot of abundance of organisms found over the course of 12 weeks, beginning on July 9, 2022. Control plots are shown in red and experimental plots are shown in blue.

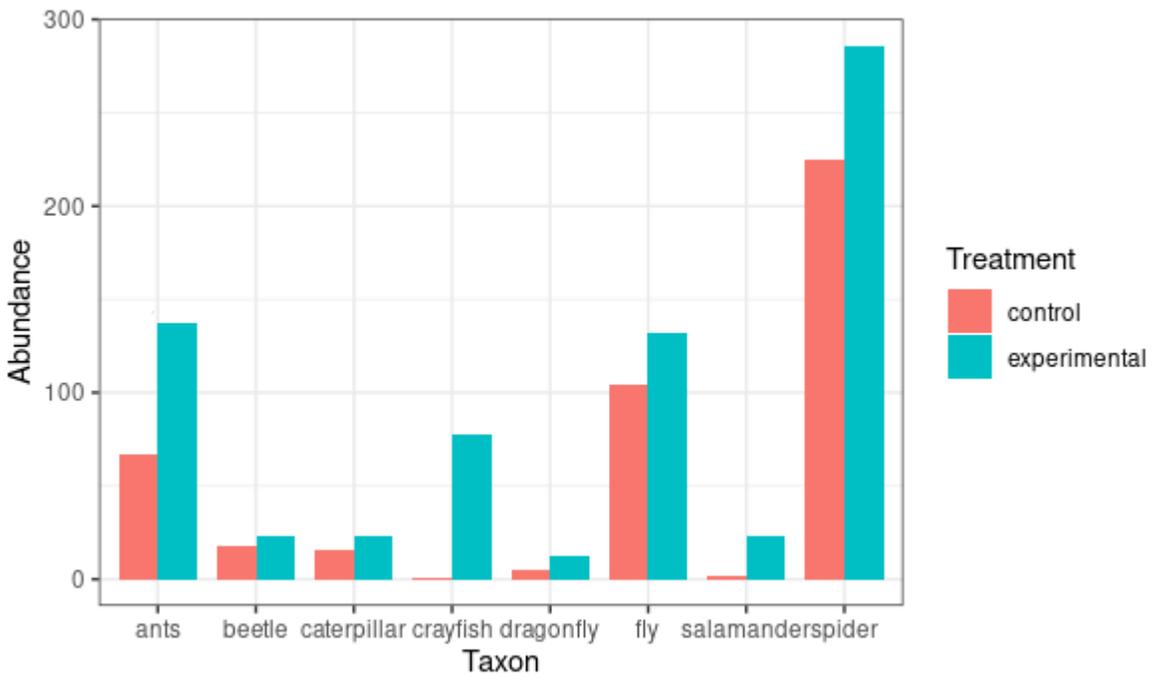


Figure 4. Histogram showing the abundance of the 8 different taxa found for experimental (blue) and control (red) plots. Crayfish and ants had a significant difference in Tukey HSD test, $p < 0.05$.

3.2. Taxon Richness

Both date and treatment had significant effects on taxon richness (Table 1). The effect of treatment did not differ across dates. Like abundance, taxon richness peaked around weeks 5 and 6 and was lowest at week 12 (Figure 4). Over the course of this study, animals were categorized into 8 taxa. Animals that did not fit into these taxa were listed as “other”. The “other” group was excluded from the analysis because the organisms were unable to be identified. Although eight different taxa were seen at various points throughout this study, plots only had at most 7 different taxa occurring in them during any week of the study. The number of taxa found per plot fluctuated over the course of the study (Figure 5). The number of taxa occurring in both experimental and control plots was highest at the beginning and middle weeks of the study (weeks 1-6) with around 6 taxa total, and gradually decreased from weeks (7-12) to around 2 taxa.

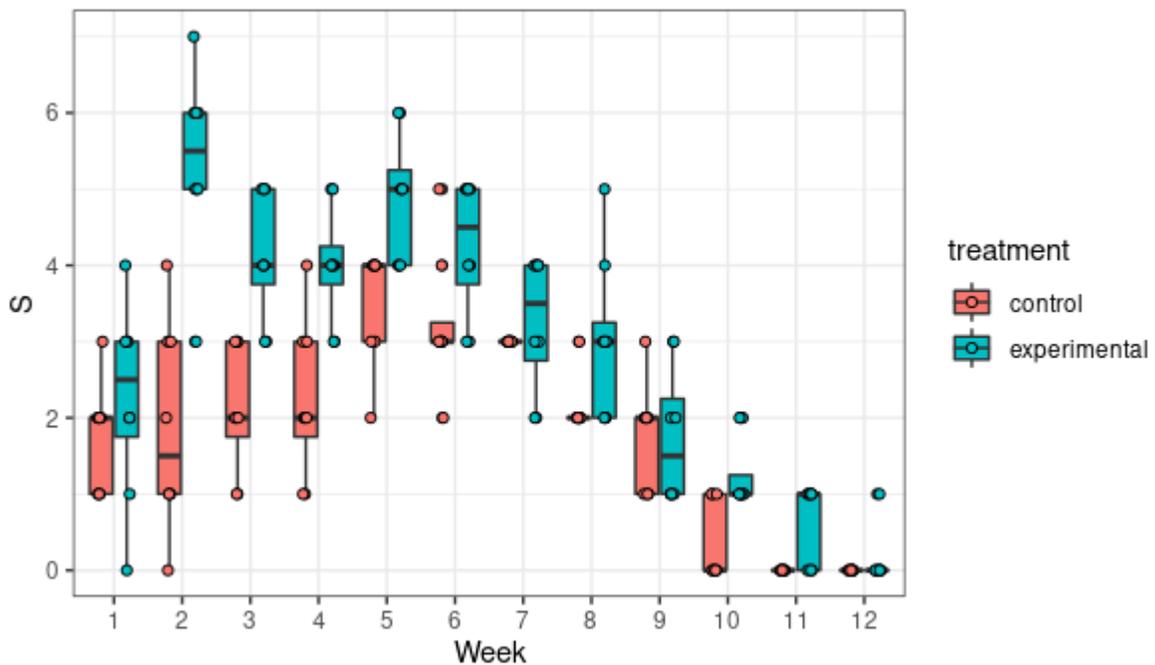


Figure 5. Boxplot of species richness over the course of 12 weeks, beginning on July 9, 2022. Control plots are shown in red and experimental plots are shown in blue.

3.3. Community Composition

A Principal Component Analysis (PCA) was used to visualize the community as a whole within experimental and control plots (Figure 6). Experimental plots had both higher abundance and higher species richness than control plots (Table 1), but their taxonomic composition was generally the same, as indicated by the overlap in the PCA plot (Figure 6). Variation in PC1, which explained 27% of the total variance among plots (Table 2), was primarily driven by the abundance of crayfish, ants, flies, and spiders. Variation in PC 2 (17% of variance) was driven by the abundance of flies, spiders, dragonflies, beetles, and salamanders (Table 3).

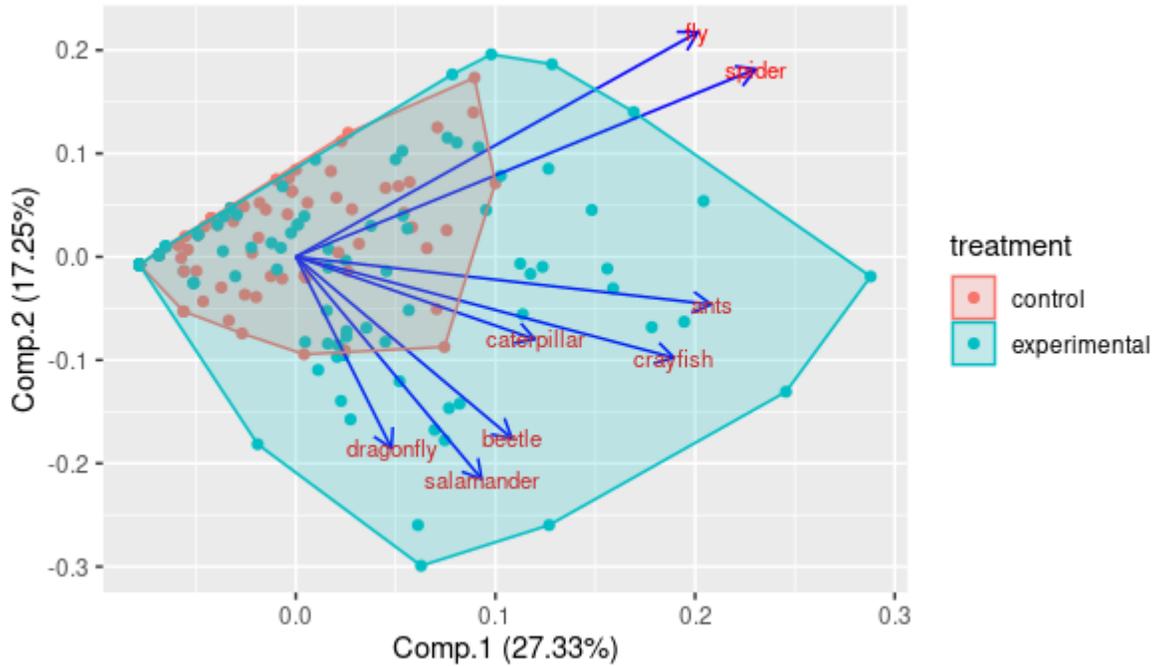


Figure 6. PCA analysis showing 8 taxon categories and the effects of experimental (blue) and control plots (red).

Table 1. Table of SRH results for taxa abundance by treatment, species richness by treatment, and total abundance by treatment

	DF	Sums of Squares	H	p
Individual taxa abundance by treatment				
Taxon	8	66490944	425.8	< 0.001
Treatment	1	4952283	31.7	< 0.001
Taxon x Treatment	8	6534663	41.9	< 0.001
Taxon richness by treatment				
Date	11	372606	124.4	< 0.001
Treatment	1	52107	17.4	< 0.001
Date x Treatment	11	34410	11.5	0.4
Total abundance by treatment				
Date	11	437277	142.9	<0.001
Treatment	1	33101	10.8	0.001
Date x Treatment	11	17086	5.6	0.899

Table 2. Table of standard deviation of proportion of variance in communities (taxa) occurring in control and experimental plots.

	Comp 1	Comp 2	Comp 3	Comp 4	Comp 5	Comp 6	Comp 7
SD	1.37	1.17	0.96	0.94	0.90	0.88	0.59
Proportion of Variance	0.27	0.20	0.13	0.13	0.12	0.11	0.05
Cumulative Proportion	0.27	0.47	0.60	0.72	0.72	0.95	1.00

Table 3. Loadings of each of the 8 different taxa in the measurements of principal components.

	Comp 1	Comp 2	Comp 3	Comp 4	Comp 5	Comp 6	Comp 7	Comp 8
Spider	0.503	0.396		0.114	0.106	0.204		0.718
Fly	0.440	0.475		0.210	0.261	0.112	-0.172	-0.653
Caterpillar	0.261	-0.174	0.782	0.190		-0.492		
Beetle	0.236	-0.383	-0.168	-0.289	0.782	-0.262		
Crayfish	0.415	-0.214	-0.280	-0.219	-0.430		-0.681	
Salamander	0.213	-0.470	0.344	-0.102		0.771		-0.109
Dragonfly	0.102	-0.405	-0.334	0.842				
Ant	0.454	-0.101	-0.226	-0.237	-0.338	-0.183	0.700	-0.197

4. Discussion

4.4. Taxa abundance

Animal abundance for the eight taxa was higher in experimental plots, than in control plots regardless of date. The most common taxon found in plots was spiders with the second most common being flies. Flies are a type of prey on which predators like spiders feed²⁰. This may be an example of the predator-prey interactions happening between

spiders and flies. Results from the Tukey HSD test found that both ants and crayfish occurred at significantly higher numbers in experimental plots than in control plots. No other taxa had significantly different abundance in control plots compared to experimental plots. Crayfish do require water, so these results could indicate that crayfish are seeking out the water-filled depressions created by the auger hole. If crayfish are seeking refugia in these holes, this may indicate that crayfish populations may benefit from cattle grazing. The high ant numbers in experimental plots was a surprising find, as ants are not known for requiring much water.

The date also had a significant effect on the overall abundance of animals in plots. Abundance was highest during weeks 5 and 6 (8/6/22 and 8/13/22) and lowest during week 12 (9/22/22). This could be due to a few reasons, including differences in temperature. July and August are the hottest months of the year for North Carolina²¹, and warm temperatures could lead to more species being active and seen by the observer. Later in the season, as temperatures drop, animals may become scarce and less likely to be seen. Another reason animal abundance may have peaked around this time is due to the life cycle of these animals. Many invertebrates breed in the summer when it is warm, and die off when temperatures start to drop later in the season. Crayfish are abundant in the summer but burrow underground during the fall and winter^{22,23}, which could explain why crayfish were not seen by the observer during the last few weeks of this study.

4.5. Taxon richness

During this study animals were organized into 8 different taxa, along with a group called “other” which included unidentified animals. Taxon richness was higher in experimental plots than in control plots throughout this study. This suggests that many different taxa are attracted to plots with auger holes. It may also be inferred that if many different animal taxa are attracted to water-filled holes created by an auger, then they may also be attracted to water-filled depressions created by cattle moving through bog habitat. Although 8 different taxa were found over the course of this study, plots never had more than 7 taxa found in them during any given point and the number of taxa found in plots varied significantly with the date observations were taken. The date had a significant effect on the taxon richness. Richness peaked for both experimental and control plots during weeks 5 and 6 (8/6/22 and 8/13/22) and was lowest during week 12 (9/22/22). This could be due to a number of factors. Firstly, the warm temperature in August could explain why more taxa were seen during weeks 5 and 6 and colder temperatures could explain why fewer taxa were found during week 12.

Another explanation could be that near the end of the study, the water-filled depressions created by the auger had started to fill in with mud and were no longer acting as refugia for water-dependent species such as crayfish²⁴. If this is the case, then it makes sense that the later weeks have lower taxa richness in experimental plots, yet throughout the course of the study experimental plots still had higher taxa richness than control plots. If mud filling in the holes was the only reason why experimental plots had less richness near the end of the study than at the beginning, then the later weeks should have similar taxa richness for both experimental and control plots, yet that is not the case. It is more likely a combination of temperature, season change, animal life cycles, mud filling in holes, and many other unknown factors influenced the taxa richness throughout the 12-week course of this study.

4.6. Community Composition

A Principal Component Analysis (PCA) was used to determine the community composition of both experimental and control plots. The taxonomic composition was the same in both experimental and control plots; however, experimental plots had both higher abundance and higher species richness than control plots. Due to the close proximity of experimental plots to control plots, it is not surprising that both plot types had similar communities of animals within them. Variation in PC1, which explained 27% of the total variance among plots, was primarily driven by the abundance of crayfish, ants, flies, and spiders. Variation in PC 2 (17% of variance) was driven by the abundance of flies, spiders, dragonflies, beetles, and salamanders. All eight taxa occurred in both types of plots, but taxa numbers fluctuated week to week in all plots. Experimental plots had a higher variation of what taxa occurred

during different weeks of the study than control plots. This could correlate to the higher taxa richness and abundance seen in experimental plots. These results could be explained by animals seeking out the augur holes found in experimental plots. This test did not take into consideration the date, which could have influenced the results as the date had a significant effect on both the abundance and taxon richness of plots.

4.7. Conclusions

Experimental plots with augur holes consistently had significantly higher taxon richness and animal abundance than control plots. This suggests that animals are seeking out the water-filled depression created by the augur. It can be inferred that in wetlands and bogs with cattle present, animals also seek out the hoof depressions created by cattle. Understanding more about the effects cattle have on bogs has important implications for conservation work. Due to how rare bog habitat is and how many rare and threatened species rely on this habitat, conserving these habitats is crucial^{24, 25}. Having a better understanding of some of the effects cattle have on ecosystem structure is also important to ecologists and conservationists as most bogland in North Carolina occurs on private land where cattle range.

Although care was taken to work in the least intrusive way possible during this study, the approaching researcher may have scared some animals away from plots during the day's observations were taken. If this study were to be replicated, stop motion cameras could be useful to supplement observations made in the field in a less intrusive way. Many other factors such as weather, time of day, and temperature may also have had effects on abundance and taxon richness. Future research in this area could include looking at how microscopic animal communities are affected by stimulated cattle hoof depressions, and other effects cattle may have on bog ecosystems

5. Acknowledgments

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