Spring 2019

# MS Environmental Biology Capstone Project

Natalie Moreno

# MS ENVIRONMENTAL BIOLOGY
# CAPSTONE PROJECT

by

Natalie N. Moreno

A Project Presented in Partial Fulfillment
of the Requirements for the Degree
Masters of Science
in Environmental Biology

REGIS UNIVERSITY
May, 2019

MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

Natalie N. Moreno

has been approved

May 2019

APPROVED:

_____________________________________, Kristofor Voss, Ph.D (Faculty Advisor)

_____________________________________, Kristofor Voss, Ph.D. (Chapters 1 & 2)

_____________________________________, Michael Ennis, Ph.D. (Chapter 3)

_____________________________________, Anna Braswell, Ph.D. (Chapter 4)

_____________________________________, Ariel Wooldridge, M.S. (Exit Survey & Repository)

# Table of Contents

# FIGURE AND TABLE LIST

## CHAPTER 2, LIST OF TABLES



## CHAPTER 2, LIST OF FIGURES



## CHAPTER 3, LIST OF FIGURES

# CHAPTER 1: LITERATURE REVIEW

## Macroinvertebrates a Reliable Indicator of Success in Restoration

### Introduction

The saying "If you build it, they will come" comes from the movie *Field of Dreams*, but also is a hypothesis in the restoration literature that assumes habitat enhancement or creation restores biotic integrity (Sudduth et al., 2011). In this review I seek to answer the following questions: (1) Under what circumstances do macroinvertebrates reliably indicate success of instream restoration projects? and (2) Why does the macroinvertebrate community fail to recover despite improvement of habitat?

Common goals for river restoration in the United States are to 1) enhance water quality, 2) manage riparian zones, 3) improve in-stream habitat, 4) allow for fish passage, and 5) stabilize banks (Bernhardt et al., 2005). Unfortunately, the majority of moderate size restoration projects in the US are not sufficiently tracked. Thus, leaving future practitioners with the responsibility to gather and publish data on the success of different restoration methods (Bernhardt et al., 2005).

Within the last decade there have been over 6,000 instream habitat enhancement projects, which exceed $1 billion (Miller et al., 2010). This could be why we need to assess the consistency of responses, and the factors explaining project performance (Miller et al., 2010). Since 1990, there has been an increase in individual stream restoration project costs. Finding the most effective way to restore a stream to its desired ecological standing can minimize the amount of spending on restoration projects (Alexander and Allan, 2006). One of the biggest issues with restoration projects is the inconsistency of pre- and post-project measurement. Only 15-30% of restoration projects include post-project monitoring (Miller et al., 2010). While many do not measure the conditions of the environment before restoration (Bernhardt et al., 2005), in some cases, measurements post-restoration are not even collected or practitioners assess the restoration site as little as one year post-restoration. The lack of pre-restoration data, or having an insufficient amount of time between restoration and project assessment can lead to an invalid assessment of the restoration projects success. An invalid conclusion for a restoration project can imply that the methods used to restore the stream failed, or that the indicator species used to measure success wasn’t the right one.

Success in river restoration projects has three primary axes: ecological success, stakeholder success, and learning success (Palmer et al., 1997). Some will argue that to measure the success of river restoration, one only needs to focus on ecological factors, whereas others will argue that one must also look into the historical, social, cultural, political, esthetic, and moral aspects (Wortley et al., 2013). Standardized ways to measure the success or failure of a restoration project are still not available, but many practitioners use aquatic organisms to quantify success (Jähnig et al., 2011). Having knowledge on how and when different aquatic species use different parts of a stream network is required for a successful restoration project (Roni et al., 2002).

Different restoration methods may show different recovery rates of change in biota. The main goal behind every instream restoration project is to increase the diversity, density, and biomass of aquatic organisms by improving hydraulics, substrate heterogeneity, and food availability (Miller et al., 2010). Restoration practitioners commonly use the presence and diversity of macroinvertebrates to indicate whether a restoration project has met its ecological goals. Changes in presence/absence, abundance, morphology, physiology, or behavior of these organisms’ can indicate that the physical and/or chemical conditions in a stream are outside the organisms preferred conditions (Resh and Rosenberg, 1993). The presence of numerous highly tolerant organisms usually indicates poor water quality (Hynes, 1998). Macroinvertebrates are a useful way to measure success in instream restoration projects and are believed to be reliable indicators of the ecological status in streams (Nilsson et al., 2015).

Habitat degradation is a threat to biodiversity, so understanding the conditions under which habitat restoration successfully impacts macroinvertebrates is important because it can tell us several things about the environment in which the macroinvertebrates live (Miller et al., 2010). Knowing which are present before and after restoration can establish whether the end goal of the restoration plan was met. Evaluating different restoration methods and how they used macroinvertebrates in their projects indicate if macroinvertebrates are a sufficient way to measure success, and if they are not, then what is?

Under certain conditions, for example when an appropriate amount of time for recovery has passed, macroinvertebrates can be used to reliably track success of instream restoration. In some scenarios, macroinvertebrates can indicate success sooner than other assemblages. Slow recovery rate is typically caused by intense restoration methods. Although some projects take longer than others to indicate success or failure, this does not imply that a restoration was a fail. In certain situations, using macroinvertebrates as an indicator species is not as effective as others. When tracking success in urban stream restorations, practitioners previously suggested that using macroinvertebrates may not be appropriate due to the size and structure difference compared to non-urban streams. Practitioners suggest that when restoration methods include intense impact that using different or multiple species may be more beneficial.  Understanding when to use macroinvertebrates as a parameter in restoration projects will save practitioners not only time but also money when deciding what to measure.

**Macroinvertebrates, a reliable way to measure success**

In a restoration project, macroinvertebrates can reveal what is considered a successful or failed project. No restoration plan is the same; projects can include removal of debris, addition of instream structures, or even water treatments. These different methods of instream restoration projects can affect the environment in different ways. Knowing if macroinvertebrates are reliable indicators across different restoration methods will give some guidelines for the conditions under which they may be used to monitor the success of these projects.

A project that was executed in a degraded rangeland creek in the Sierra Nevada showed that the macroinvertebrate community improved in as little as 2 years (Herbst and Kane, 2009). The restoration project included construction of a channel and rehabilitation of gullies and roads in the surrounding watershed. The authors measured the progress/success by sampling macroinvertebrates. From the macroinvertebrates sampling Herbst and Kane (2009), found an increase in EPT taxa (mayflies, stoneflies, caddisflies), the proportion and diversity of sensitive taxa, and consumers of riparian organic matter (shredders). The authors also noted a decrease in tolerant taxa and in fine organic particle filter-feeders (Herbst and Kane, 2009). Because of the fast recovery of the macroinvertebrate community, we can conclude that in this case, for these restoration methods macroinvertebrates are good indicators of success on a short time frame.

Not all projects see success in such a short period of time. Many streams in Finland have been channelized over the years because they are used to transport timber. Consequently, landowners and ecologists aimed to restore many streams back to their pre-channelized condition. The authors (Muotka et al., 2002) revisited and re-evaluated streams that were originally sampled by Lassonen et al. (1998). To restore these streams, boulders were added and flow detectors were installed. Macroinvertebrates were then used to evaluate the restoration progress 4-8 years post-restoration. The macroinvertebrate analysis showed long-term recovery potential for these specific restoration methods. The authors concluded that projects that include a harder impact on the stream, for example that use heavy machinery, can impair the macroinvertebrate communities short-term (Muotka et al., 2002). Due to harsh impact on the macroinvertebrate community, long term monitoring in both restored and natural streams is needed to assess whether the techniques being used to restore will enhance the recovery of macroinvertebrate communities (Muotka et al., 2002).

In restoration plans practitioners will occasionally witness success as Muotka et al. (2002) did, although that is not the case for every restoration project. In north-eastern Finland, a study examined the effects of restoration structures (addition of boulders, flow deflectors, and digging excavations) on retentive efficiency (percentage of leaves retained) in eight streams (Muotka and Laasonen, 2002). Leaf retention in streams enhances both the community structure and the metabolism of streams (Wallace et al., 1997; Crenshaw et al., 2002). By using macroinvertebrates, the authors assessed how the environment progressed post restoration. When comparing the macroinvertebrate communities between pre- and post-restoration and between naturally retentive streams, the authors noticed that the only macroinvertebrate group whose density increased significantly were algae feeding scrapers (Muotka and Laasonen, 2002). This is because the restoration methods, which included the use of heavy machinery, drastically reduced the moss cover by completely detatching the moss from the stream bed. Moss is not only important for retaining leaf litter, but also retains fine suspended particles that provide feeding arenas for macroinvertebrates (Muotka and Laasonen, 2002). After the analysis the authors concluded that the restoration had not improved retentive efficiency or macroinvertebrate community structure. Despite the authors not getting their desired outcome, macroinvertebrates were able to reliably indicate that these methods were not the most ideal for their overall ecological goals because of the impact the heavy machinery caused on the stream.

A meta-analysis comparing different restoration projects can thoroughly evaluate different methods amongst each other, whereas individual projects may have gaps. A common indicator species during restoration projects are macroinvertebrates, they can show whether certain restoration methods are better than others. Since macroinvertebrates can successfully indicate success in instream projects, a meta-analysis synthesized 18 different case studies that tracked macroinvertebrate responses to in-stream habitat restoration plans in Scandinavian streams (Nilsson et al., 2015). This meta-analysis examined how effective macroinvertebrates were at successfully indicating restoration plan success. The addition of large woody debris and boulders improved macroinvertebrate richness, but the addition of boulders did not consistently. The inconsistent measures from the additions of boulders, results from boulders disrupting the macroinvertebrate communities when being placed within the stream. The authors concluded that across all the studies the meta-analysis displayed an increase of macroinvertebrate richness. This supported Nilsson et al. (2015), hypothesis that an increase of habitat heterogeneity can enhance macroinvertebrate richness.

Stream restoration projects differ in their goals. Since not every restoration project is the same, the techniques used across many projects will always be different. In the Arkansas River, a metal contaminated stream in Colorado, USA over a 17-year period, different variables were measured to assess restoration success over 17 years (Clements et al., 2010). In many restoration projects instream structures are added to reduce erosion or to return a river to its original physical condition. However, in the Arkansas River, restoration aimed to treat water quality. In order to restore the stream, water treatment facilities were installed on the Leadville Mine Drainage Tunnel and the Yak Tunnel. These two facilities treated the metal contaminated water to improve water quality (Clements et al., 2010). At one of the sites, the authors also incorporated the removal of mine tailings and revegetation of riparian areas. When assessing whether the treatment facilities improved the water quality Clements et al. (2010) sampled macroinvertebrates. The authors quantified macroinvertebrate abundance and mayfly richness

(Ephemeroptera) because their previous studies showed that mayflies are especially sensitive to metals (Clements et al., 2010). The authors noticed that macroinvertebrates at sites closer to the water treatment facilities improved faster than sites further away. The authors conclude that the macroinvertebrate communities showed more improvement at sites focused purely on water quality as opposed to those that included the revegetation and the removal of mine tailings. This could be because water quality drastically impacts which taxa are present in the stream. This implies that when a habitat is directly impacted, whether it be water quality or the addition of instreams structures, the rate of recovery can vary.

**When macroinvertebrates fail to indicate success**

Macroinvertebrates are commonly used to measure success of restoration projects, but there are always those certain situations when they may not be the best parameter to follow when tracking success. Herring et al. (2015) compared the effectiveness of large-scale intensive restoration methods and smaller-scale restoration methods. In a meta-analysis ten pairs of European river sections were compared to unrestored reaches.  Habitat composition in the river and in its floodplain, three aquatic organism groups (macroinvertebrates), two floodplain inhabiting organism groups (floodplain vegetation, ground beetles), food web composition, and land water interaction were the parameters measured in this paper (Herring et al., 2015). After comparing these variables between large- and small-scale restoration plans, the authors recognized no difference in the aquatic and floodplain biota compared to unrestored streams, but they found that floodplain ground beetles positively responded to both large- and small-scale restoration plans (Herring et al., 2015).  A possible reason why ground beetles significantly improved faster is because floodplain biota were not directly impacted by restoration efforts in this case. Aquatic organisms most likely do not recover as quickly when they are directly affected by the efforts of restoration, so more time would be required for their communities to recover. Thus, floodplain biota like ground beetles, would be more likely than macroinvertebrate communities, to respond quickly to stream restoration efforts (Herring et al., 2015).

Unlike the work done by Herring et al. (2015), who included several parameters to measure the success of restoration, some studies focus specifically on one taxonomic group. In many projects, macroinvertebrates are the organisms used to indicate success, but this could be a weakness because macroinvertebrate communities may take longer to recover (Nilsson et al., 2015). When synthesizing data from 18 case studies and comparing the abiotic and biotic responses across the cases, Nilsson et al. (2015) concluded that macroinvertebrates are poor indicators of ecosystem response to restoration (Nilsson et al., 2015). Throughout the 18 case studies, methods of restoration included returning coarse sediment and sometimes woody debris to the reach. Using these methods drastically impacted the macroinvertebrate community causing disturbance and detaching moss resulting in an overall decrease in moss coverage. Using macroinvertebrates to study the success of these restoration methods is not ideal. During restoration projects, one shouldn't focus on a single taxonomic group variable because Nilsson et al. (2015) suggest that using a wider range of organisms could be more beneficial than focusing solely on one taxonomic group.

Macroinvertebrates are unreliable indicators both when restoration methods disrupt the sediment of the streams, and when restoration occurs in urban streams. In Northern California a small urban stream was restored to open a previous culverted channel. Other restoration methods included planting vegetation and adding in-stream structures to improve water quality and vegetation within the riparian zone (Purcell et al., 2002). The authors sampled macroinvertebrates within their adult stages to compare taxa richness between restored and unrestored sites. Purcell et al. (2002) perceived that most taxa were (12 out of 13) recolonizers and did not live in the restored site within their immature aquatic stages. Only one species inhabited the creek's waters post-restoration. The authors are uncertain whether habitat or water quality conditions have prevented other species from re-establishing, or whether they are present but too low to detect. Because of this finding Purcell et al. (2002), believes that using metrics from non-urban streams is not ideal when assessing restoration success in urban waterways (Purcell et al., 2002).

## Conclusion

There is no standardized way to measure the success of restoration projects, but there are sources and previous work that can tell restoration practitioners which parameters may accurately measure success. Using a reliable way to monitor success, whether it be macroinvertebrates, or another indicator species can help conserve the millions of dollars being spent on stream restoration projects. This review examined those circumstances under which macroinvertebrates may reliably indicate success of instream restoration projects, but also why the macroinvertebrate community may fail to recover despite habitat restoration.

Time since restoration is considered one of the most important factors that correlates with macroinvertebrates being a reliable indicator of success. In some projects, practitioners will not see success for many years, whereas others see it in as little as one year. This discrepancy in recovery time across projects may be due to the degree of impact that occurs during the restoration project itself. Restoration activities with minimal impact may recover quickly while those with intense impact may take more time. Lack of fast recovery however does not imply that macroinvertebrates fail to indicate success in a project. Rather it could just imply that long-term monitoring of macroinvertebrate communities is required. For example, when restoration activities use heavy machinery or restore coarse sediment macroinvertebrates are not an ideal parameter but can still indicate success long-term.

Even so, some methods of restoration witness improvement in macroinvertebrates while others do not. The findings from this review also suggest that when the water quality is directly improved by treating pollution, the change in macroinvertebrate communities can reliably indicate success or failure of a restoration project. Occasionally macroinvertebrates will not be the best way to monitor success. When intense restoration takes place, and the macroinvertebrate community is heavily impacted, practitioners suggest that other species/organisms may be a better parameter to monitor success. Previous studies also suggest that when evaluating an urban stream, that one should not use the metrics that are commonly used in non-urban streams. Using the same metrics between urban and non-urban streams is not valid because of the drastic size and composition difference between the streams.

Since many previous restoration projects failed to adequately record and publish data on the success of their restoration projects, future practitioners are unsure what the best approach may be to maintain their projects' success. Stream restoration plans have become progressively common and the demand for post-project monitoring is clear. With billions of dollars being spent on stream restoration projects annually, the findings from this review can help mitigate annual expenditures. Whether it be macroinvertebrates or another indicator species, understanding what reliably indicates success when choosing a monitoring method for a project will save not only money but also time.

**Literature Cited**

Alexander, G. G., & Allan, J. D. (2006). Stream restoration in the Upper Midwest, USA. *Restoration Ecology*, *14*(4), 595-604.

Bernhardt, E. S., Palmer, M. A., Allan, J. D., Alexander, G., Barnas, K., Brooks, S., ... & Galat,D. (2005).Synthesizing US river restoration efforts.

Clements, W. H., Vieira, N. K., & Church, S. E. (2010). Quantifying restoration success and recovery in a metal-polluted stream: A 17-year assessment of physicochemical and biological responses. *Journal of Applied Ecology*, *47*(4), 899-910.

Herbst, D. B., & Kane, J. M. (2009). Responses of aquatic macroinvertebrates to stream channel reconstruction in a degraded rangeland creek in the Sierra Nevada. *Ecological Restoration*, *27*(1), 76-88.

Hering, D., Aroviita, J., Baattrup-Pedersen, A., Brabec, K., Buijse, T., Ecke, F., ... & Kupilas, B. (2015). Contrasting the roles of section length and instream habitat enhancement for river restoration success: A field study of 20 European restoration projects. *Journal of Applied Ecology*, *52*(6), 1518-1527.

Hynes, K. E. (1998). Benthic macroinvertebrates diversity and Biotic indeces for monitoring of 5 urbanizing lakes within the Halifax regional municipality (HRM), Nova Scotia, Canada. *Soil and Water Conservation Society of Metro Halifax, 114p*.

Jähnig, S. C., Lorenz, A. W., Hering, D., Antons, C., Sundermann, A., Jedicke, E., & Haase, P. (2011). River restoration success: a question of perception. *Ecological Applications*, *21*(6), 2007-2015.

Miller, S. W., Budy, P., & Schmidt, J. C. (2010). Quantifying macroinvertebrate responses to in stream habitat restoration: applications of meta-analysis to river restoration. *Restoration Ecology*, *18*(1), 8-19.

Muotka, T., & Laasonen, P. (2002). Ecosystem recovery in restored headwater streams: The role of enhanced leaf retention. *Journal of Applied Ecology*, *39*(1), 145-156.

Muotka, T., Paavola, R., Haapala, A., Novikmec, M., & Laasonen, P. (2002). Long-term recovery of stream habitat structure and benthic invertebrate communities from in-stream restoration. *Biological Conservation*, *105*(2), 243-253.

Nilsson, C., Polvi, L. E., Gardeström, J., Hasselquist, E. M., Lind, L., & Sarneel, J. M. (2015). Riparian and in-stream restoration of boreal streams and rivers: Success or failure? *Ecohydrology*, *8*(5), 753-764.

Palmer, M. A., Ambrose, R. F., & Poff, N. L. (1997). Ecological theory and community restoration ecology. *Restoration ecology*, *5*(4), 291-300.

Purcell, A. H., Friedrich, C., & Resh, V. H. (2002). An assessment of a small urban stream restoration project in northern California. *Restoration Ecology*, *10*(4), 685-694.

Roni, P., Beechie, T. J., Bilby, R. E., Leonetti, F. E., Pollock, M. M., & Pess, G. R. (2002). A review of stream restoration techniques and a hierarchical strategy for prioritizing restoration in Pacific Northwest watersheds. *North American Journal of Fisheries Management*, *22*(1), 1-20.

Resh, V. H., & Rosenberg, D. M. (1993). *Freshwater biomonitoring and benthic macroinvertebrates* (No. 504.4 FRE).

Sudduth, E. B., Hassett, B. A., Cada, P., & Bernhardt, E. S. (2011). Testing the field of dreams hypothesis: functional responses to urbanization and restoration in stream ecosystems. *Ecological Applications*, *21*(6), 1972-1988.

Wortley, L., Hero, J. M., & Howes, M. (2013). Evaluating ecological restoration success: a review of the literature. *Restoration Ecology*, *21*(5), 537-543.

# CHAPTER 2: GRANT PROPOSAL

The Effect of Beetle Biodiversity on Dung Decomposition at Rio Mora National Wildlife Refuge (RMNWR)

**Section 1: Abstract**

Decomposers are the finishing consumers of organic matter. Specifically, dung beetles (Coleoptera: Scarabaeidae) are well known decomposers that are keystone species in grasslands because they decompose dung, disperse seeds, and enrich the soil with nutrients. Within the RMNWR and neighboring ranches, bison and cattle produce dung which must be decomposed by dung beetles. Consequently, understanding how dung beetle diversity affects dung decomposition is vital to ensure that the use of bison as ecosystem engineers at RMNWR does not overburden the grassland with waste. Therefore, I propose to sample dung beetles at RMNWR and a nearby cattle ranch to compare the composition of the assemblage at these two sites. I will then experimentally assess how dung beetle richness affects the decomposition rate of dung and whether dung beetles decompose dung as generalists or as specialists. The results from this study will provide baseline data on dung beetles in the grassland ecosystem and the degree to which they decompose dung. It will also provide insight for future studies on how biodiversity plays a significant role with ecosystem functioning.

**Section 2: Literature Review, Objective, Anticipated Value, Question and Hypothesis**

**Literature Review**

Producers, consumers, and decomposers are three biotic components of an ecosystem. Specifically, decomposers are the finishing consumers of organic matter by breaking down other organisms' wastes. (Heitschmidt et al., 1996). Dung beetles (Coleoptera: Scarabaeidae), a well-known decomposer of organic matter, are considered keystone species in ecosystems because of the disproportional role they play in comparison to their abundance (Numa et al., 2012). Dung beetles decompose dung, disperse seeds (Andresen and Levey, 2004), and control parasites that infest vertebrates (Horgan, 2005). By decomposing dung, dung beetles save the US cattle industry approximately $380 million dollars annually (Losey and Vaughan, 2006) by enriching soil with organic matter thereby reducing the need for artificial fertilizers (Stevenson and Dindal, 1987).

Dung beetles comprise three main functional groups, each of which uses different mechanisms to break down dung: (1) *paracoprids*, which dig tunnels and build their nests directly beneath the dung mass; (2) *endocoprids*, which construct a nest cavity within the dung; and (3) *telecoprids*, which detach a portion of dung from the mass, roll it some distance away from the dung then bury it (Numa et al., 2012). Since the species within these groups have different roles, their processing rates may differ according to environmental conditions. Endocoprids are more effective in open areas, specifically in dry land with high temperature (e.g., compact, dry) (Numa et al., 2012), whereas telecoprids and paracoprids need specific soil characteristics (e.g., loose, moist) for burying the dung and constructing nests (Yamada et al., 2007). When a habitat undergoes change that affects soil composition, biodiversity within the assemblage will decrease which could negatively affect the decomposition rate of dung.

Although dung beetles vary in their different functional traits, as generalists they thrive across a range of environmental conditions and facultatively use different resources. However, when resources are scarce, dung beetles can specialize on the type of dung they inhabit (Estrada et al., 1993). In these instances, their preference for dung varies according to the condition of dung (e.g., dry, moist, fresh, old) (Doube, 1987) and the odor of dung (Dormont et al., 2004). Other key environmental factors that influence the composition of dung beetle assemblages are dung type, soil type, and habitat type (Numa et al., 2012).  Factors that affect soil such as vegetation structure, forest canopy cover, human habitat modification, and forest fragmentation, also influence the abundance of telecoprids and paracoprids (Numa et al., 2012; Jankielsohn, 2001).

Changes to the dung beetle assemblage brought about by habitat modification may also influence the rate at which dung is processed. Such association between biodiversity and ecosystem function is of growing significance in ecology (Griffiths et al., 2000). Two theories that explain the increase in ecosystem functioning from a more diverse mixture of species are: (1) the selection effect, which occurs when a dominant species with favorable traits is especially good at using resources, and (2) the complementarity effect, which is when resource partitioning or positive interactions between species result in an increase of total resource use (Loreau and Hector, 2001). The majority of biodiversity experiments, however, have been focused on above-ground species rather than soil organisms.

Since soils contain the highest level of diversity in organisms, this assemblage is key to maintaining ecosystem function (Griffiths et al., 2000). Nichols et al. (2008) described the different ecological roles dung beetles play, and how the functions depend on these different roles present in the assemblage. For example, telecoprids and paracoprids both dig tunnels either some distance from the dung or below the patty itself. Through this process they disperse seeds and consume dung. On the other hand, endocoprids consume dung differently by building nest cavities within the patties and therefore contributes to parasite suppression (Nichols et al., 2008).

Thus, understanding how dung beetle assemblages influence the rate of decomposition is important in managed ecosystems such as the RMNWR and other rangelands. Previous studies on dung decomposition have focused primarily on cattle dung. The findings from this project will broaden what is known about the difference in dung decomposition by comparing cattle and bison dung decomposition rates. This study differs from other dung projects because it focuses on how biodiversity, and the different mechanisms of the different species affect the decomposition rate of dung.

**Objective and Anticipated Value**

The objective of this study is to catalog the dung beetle assemblages at the RMNWR and quantify the effect of biodiversity on the decomposition rate of both cattle and bison dung. The data collected from this research will provide baseline data on the dung beetle community at RMNWR, and whether dung beetle biodiversity influences the decomposition rate.

The findings from this project will benefit the RMNWR and surrounding ranchers/landowners that have cattle. Since the RMNWR use bison as grazers to restore grassland functions, they face the constant production of bison dung. Dung beetles not only save landowners millions of dollars by enriching the soil, but also contribute to the ecosystem by dispersing seeds. This study overall will inform the RMNWR how the dung on their land is being decomposed and the extent to which research on bison dung might carry over to ranchers that have cattle.

**Questions, Hypothesis, and Predictions**

*Question 1***:** How do dung beetle assemblages differ between cattle and bison dung?

*Hypothesis and Prediction 1***:** Different dung beetles are attracted to different dung types (e.g., odor, surrounding vegetation, different species). If cattle dung and bison dung differ in their traits, then I expect the dung beetle assemblages between cattle and bison dung to differ.

*Question 2:* How does biodiversity influence the decomposition rate of dung?

*Hypothesis and Prediction 2:* Different dung species use different mechanisms to decompose dung that can directly influence the rate of decomposition. Because each species breaks down dung in different ways, I expect patties with a higher biodiversity of dung beetles to decompose dung at a faster rate.

*Question 3:* Can the dung beetle assemblages found in the bison dung at the RMNWR decompose cattle dung at the same rate they decompose bison dung?

*Hypothesis and Prediction 3:* Since dung beetles are generalists and will adapt to decompose any dung, I do not expect decomposition rates to differ in a transplant experiment where bison beetle assemblages are placed within cattle dung and cattle beetle assemblages are placed within bison dung.

## Section 3: Methods and Negative Impacts

### Methods

*Specific Aim 1(See Q1 above): Quantifying dung beetle assemblages in bison and cattle patties:*

At the beginning of spring 2019, the bison herd from RMNWR and a cattle herd from a neighboring cattle ranch will be followed to locate 15 fresh patties. Each patty will be flagged and GPS coordinates will be recorded so that each patty can be easily located seven days later (Strong et al., 1996). Around each patty pitfall trapping will be used to obtain roller dung beetles (Beynon et al., 2012). I plan on placing five pitfall traps 15 cm from the patty in a pentagonal pattern to capture telecoprids beetles. Patties will be protected with a barrier created from mesh and wooden stakes one foot above the patty to prevent foraging from birds and other mammals (Beynon et al., 2012). After seven days I will return to each site to obtain the patty and all specimens inside the pitfall traps. For each trapping location I will identify dung beetles to species both from pitfall traps and the patty using *Beetles The Natural History and Diversity of Coleoptera*. I will perform a nonmetric multidimensional scaling ordination and use permutational multivariate analysis of variance to test whether the assemblage composition differ by dung type.

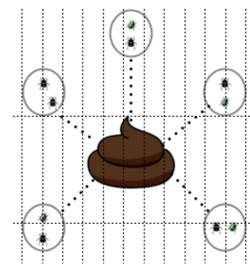

Figure 1: Telecoprid beetles will be collected from pitfall traps (15 cm from dung) and dung to obtain baseline data

*Specific Aim 2 (See Q2 above) Quantifying the effect of dung beetle biodiversity on the decomposition rate:* I will follow both the bison and the cattle herd to obtain 15 fresh patties per herd, and collect two five gallon buckets of soil from each ecosystem. Once the fresh patties are collected, I will mix separately cattle and bison patties into a uniform mixture of each type. Then, I will weigh out 20 1-kg samples of dung from both dung types (Yamada et al., 2007). Each separate sample will be placed in a 10-gallon aquarium that has a compact four-inch layer of soil collected from the grassland where dung was collected. Dung samples will be treated with cattle and bison specific beetle assemblages from Specific Aim 1. The treatments will consist of 40 dung beetles of the same species, 40 dung beetles of three different species, 40 dung beetles of five different species, and a control without beetles (Yamada et al., 2007). The specific number of species may change depending on the species observed in Specific Aim 1. The specific species composition of each treatment will be randomly selected such that each beetle's species is represented in at least 3 replicates. The control group will consist of five patties of both bison and cattle dung with no dung beetles present. After seven days of placing the beetles within the aquariums, I will remove the dung beetles from the dung and weigh the dung to calculate the decomposition rate and construct a linear regression to assess the effect of biodiversity on decomposition (Yamada et al., 2007).

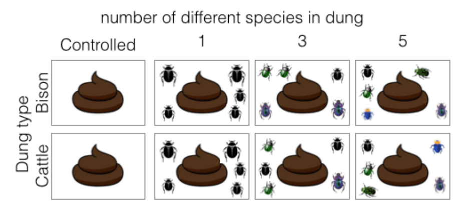


Figure 2: Different species will be placed in different dung samples to observe how biodiversity affects

*Specific Aim 3 (See Q3 above) Assessing beetle specificity on dung:* Again, both herds will be followed as above to obtain nine fresh patties so that pre-weighed samples of dung can be placed inside 10-gallon aquariums with four-inch layers of soil. In six aquariums that contain cattle dung I will place 40 randomly selected individuals of 3 species that were collected from the bison dung during Specific Aim 1 in each

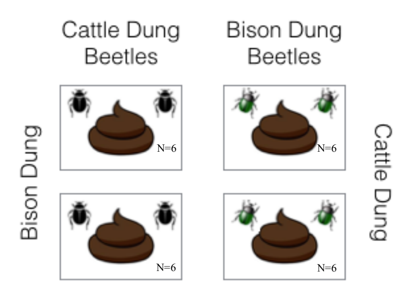


Figure 3: Species collected in cattle dung will be placed within the bison dung, and species collected within bison dung will be placed in

aquarium. I will repeat this for the bison dung, placing 40 individuals of these species collected from cattle dung into aquariums containing bison dung. The remaining three aquariums for each patty will be a control, consisting of three patties from both the bison and cattle herd with individuals that originally came from that specific patty type. I will collect the specimen and weigh to compare cattle and bison dung decomposition rates using a two-way ANOVA (Yamada et al., 2007).

**Negative impacts:**

Throughout this project I will be obtaining dung patties and dung beetle individuals which will both have minimal impact due to their high abundance.

## Section 4: Timeline and Budget

### Timeline

| Dates | Activities | Deliverables |
|---|---|---|
| Late April 2019 - Early May 2019 | • Deploy traps on cattle and bison dung<br>• Collect beetles<br>• Perform NMDS | • Raw data from surveys<br>• Baseline data on what species are present across bison and cattle dung<br>• A graphical figure clustering dung beetle species<br>• Species collection for specific aim two and 3/ Experimental design for specific aim 2 and 3 |
| Early May 2019 - Late May 2019 | • Obtain 15 fresh patties per herd/collect soil<br>• Set up experiment for specific aim 2<br>• Begin biodiversity experiment/Collect data | • Raw data from surveys<br>• Soil collected for specific aim 2 and 3<br>• First rough draft for analysis on specific aim 1 |
| Early June - Late June | • Obtain nine fresh patties per herd<br>• Set up for specific aim 3/Begin transplant experiment<br>• Begin write up for specific aim 2/Collect data | • Raw data from surveys<br>• Analysis from raw data obtained from specific aim two |
| Early July | • Final data analysis for specific aim 3 and begin write up | • Analysis for specific aim three |
| Late August | • Complete rough draft | • Draft report |
| Late September | • Complete final write up | • Final report |

### Budget

| Item | Justification | Cost, unit (source) | Quantity | Total cost |
|---|---|---|---|---|
| **Aquarium (10 gallon) | To study decomposition rate of dung in a lab | 23.50/1 (Amazon) | 20 | 0 |
| Pitfall traps | To ID and capture rollers near patties | 12.95/10 traps (Carolina Biological Supply Company) | 30 | 38.85 |
| Ethanol | For the pitfall traps | 6.40/500 mL (Carolina Biological Supply Company) | 5 | 32 |
| 5 gallon buckets | To obtain soil, and dung | 8.91/3 buckets (Home Depot) | 6 | 17.82 |
| Wooden stakes | To build protective structure around dung | 15.47/25 stakes (Home Depot) | 150 | 92.82 |
| Mesh netting | To build protective structure around dung | 18.98/ 630 square feet (Home Depot) | 1 | 18.98 |
| Small garden shovel | To dig holes for stake placement | 7.97/1 shovel (Home Depot) | 2 | 15.94 |
| **GPS | To record coordinates of dung patties | 84.28/1 GPS (Walmart) | 1 | 0 |

| | | | | |
|---|---|---|---|---|
| Specimen jars | For beetle collection | 11.47/12 jars (Walmart) | 48 | 0 |
| Flags | To flag patties | 8.00/100 flags (Uline) | 200 | 16.00 |
| **Scale | To weigh dung | 33.90/1 scale (Amazon) | 1 | 0 |
| Beetles The Natural History and Diversity of Coleoptera | To ID beetles | 58.61/1 book (Barnes & Noble) | 1 | 58.61 |
| **TOTAL PROPOSAL REQUEST** | | | | **313.59** |
| **ITEMS WITH ** WILL BE SUPPLIESS OBTAINED FROM REGIS UNIVERSITY** | | | | |

## Rio Mora National Wildlife Refuge Map

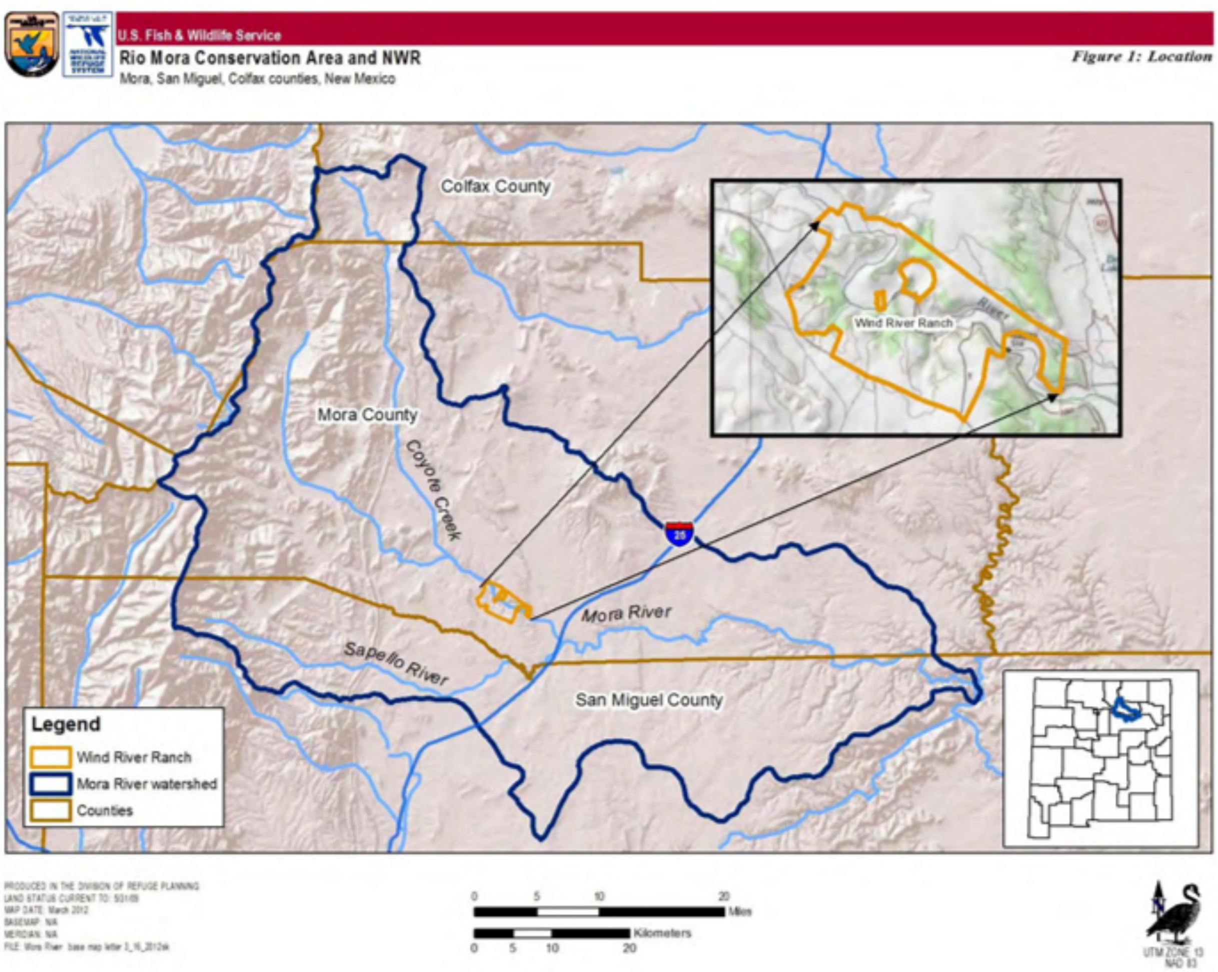


Figure 4: Rio Mora National Wildlife Refuge Map, with borders identifying the Wind River Ranch, Mora River watershed, and Counties. Within this range a nearby cattle ranch will be selected.

**Literature Cited**

Andresen, E., & Levey, D. J. (2004). Effects of dung and seed size on secondary dispersal, seed predation, and seedling establishment of rain forest trees. *Oecologia*, *139*(1), 45-54.

Beynon, S. A., Mann, D. J., Slade, E. M., & Lewis, O. T. (2012). Species-rich dung beetle communities buffer ecosystem services in perturbed agro-ecosystems. *Journal of Applied Ecology*, *49*(6), 1365-1372.

Dormont, L., Epinat, G., & Lumaret, J. P. (2004). Trophic preferences mediated by olfactory cues in dung beetles colonizing cattle and horse dung. *Environmental Entomology*, *33*(2), 370-377.

Doube, B. M. (1987). Spatial and temporal organization in communities associated with dung pats and carcasses. *Organization of Communities: Past and present*.

Estrada, A., Halffter, G., Coates-Estrada, R., & Meritt, D. A. (1993). Dung beetles attracted to mammalian herbivore (*Alouatta palliata*) and omnivore (*Nasua narica*) dung in the tropical rain forest of Los Tuxtlas, Mexico. *Journal of Tropical ecology*, *9*(1), 45-54.

Griffiths, B. S., Ritz, K., Bardgett, R. D., Cook, R., Christensen, S., Ekelund, F., ... & Dolfing, J. (2000). Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: An examination of the biodiversity ecosystem function relationship. *Oikos*, *90*(2), 279-294.

Heitschmidt, R. K., Short, R. E., & Grings, E. E. (1996). Ecosystems, sustainability, and animal agriculture. *Journal of Animal Science*, *74*(6), 1395-1405.

Horgan, F. G. (2005). Effects of deforestation on diversity, biomass and function of dung beetles on the eastern slopes of the Peruvian Andes. *Forest Ecology and Management*, *216*(1-3), 117-133.

Jankielsohn, A., Scholtz, C. H., & Louw, S. V. (2001). Effect of habitat transformation on dung beetle assemblages a comparison between a South African nature reserve and neighboring farms. *Environmental Entomology*, *30*(3), 474-483.

Loreau, M., & Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature*, *412*(6842), 72.

Losey, J. E., & Vaughan, M. (2006). The economic value of ecological services provided by insects. *AIBS Bulletin*, *56*(4), 311-323.

Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezquita, S., Favila, M. E., & Network, T. S. R. (2008). Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. *Biological conservation*, *141*(6), 1461-1474.

Numa, C., Verdú, J. R., Rueda, C., & Galante, E. (2012). Comparing dung beetle species assemblages between protected areas and adjacent pasturelands in a Mediterranean savanna landscape. *Rangeland Ecology & Management*, *65*(2), 137-143.

Stevenson, B. G., & Dindal, D. L. (1987). Insect effects on decomposition of cow dung in microcosms. *Pedobiologia*, 30, 81–92.

Strong, L., Wall, R., Woolford, A., & Djeddour, D. (1996). The effect of faecally excreted ivermectin and fenbendazole on the insect colonisation of cattle dung following the oral administration of sustained release boluses. *Veterinary Parasitology*, *62*(3-4), 253-266.

Yamada, D., Imura, O., Shi, K., & Shibuya, T. (2007). Effect of tunneler dung beetles on cattle dung decomposition, soil nutrients and herbage growth. *Grassland Science*, *53*(2), 121 129.

# CHAPTER 3: JOURNAL MANUSCRIPT

Macroinvertebrates slow recovery in a small-scale restoration project


## Abstract

One-third of the rivers within the United States are characterized as impaired or polluted, and human alteration is one of the leading causes. Because of the constant degradation of these streams, stream practitioners are spending millions of dollars restoring not only the stream but also the riparian vegetation. Here I examined macroinvertebrates response to restoration that included instream structures and the replanting of native vegetation. I predicted that the macroinvertebrate abundance in Deer Creek over the years of 2016-2018 has increased. Specifically, I predicted that the macroinvertebrate assemblage would be more abundant and diverse at stream sites that had beaver activity followed by restoration sites then lastly non-beaver activity sites. Macroinvertebrate samples, water quality data, and habitat surveys were collected across 18 sites at Deer Creek. Conflicting to the predictions the macroinvertebrate assemblages have decreased across all sites on Deer Creek. These results suggest that long-term monitoring is ideal for restoration projects, and that macroinvertebrates assemblages decrease post restoration before they reestablish.


## Introduction

More than one-third of rivers in the United States are impaired by human activities that degrade streams, such as damming, channelization, pollution discharge, and landcover change (Richardson et al., 2007). Channelization, for example, disrupts the natural flow regime by increasing the speed at which water flows through stream networks (Nilsson et al., 2015). Consequently, instream restoration projects over the last several decades have aimed to restore impaired streams to less-disturbed states (Nilsson et al., 2015). Despite this history of restoration project implementation, a general protocol to monitor post-restoration success has not been developed (Jähnig et al., 2011). Although different projects share the common goal of improving instream habitat, the precise definition of success may vary according to each project's specific goal (Miller et al., 2010). Furthermore, the rate of recovery to a less-disturbed state will also vary according to the particular restoration method used and the specific endpoint used to monitor restoration success. Regardless of these differences, biodiversity of indicator organisms is a common endpoint used in both pre- and post-restoration monitoring to assess progress of a restoration project towards its goals (Miller et al., 2010).

The macroinvertebrate assemblage is one such group of indicator organisms commonly used to track restoration progress and to assess the relative success of different restoration methods. Using macroinvertebrates as an indicator species has advantages because they are found in most water bodies, are very diverse, contribute to important ecosystem processes, and are easy to sample (Resh, 2008). Changes in presence/absence, abundance, morphology, physiology, or behavior of these organisms can imply that the physical and/or chemical condition in a stream is outside an organism's tolerance levels (Resh and Rosenberg, 1993). Herbst and Kane (2009) suggests that since macroinvertebrates respond quickly to stress that they are consistently a reliable indicator.

One main cause of stress to aquatic organisms in streams in channelization. In urban landscapes all over the world, channelization has resulted in declines to both riparian zone extent and quality (de Waal et al., 1994). By narrowing and deepening streams, channelization not only alters the stream's flow regime, but also disconnects the stream from its floodplain. For these reasons, channelization results in a loss of riparian vegetation which in turn has a dramatic and rapid effect on many hydrogeomorphic characteristics (Hupp, 1992). The biota supported by the hydrogeomorphically altered system is quite depauperate in comparison to the original system (Muotka and Syrjänen, 2007). Due to the drastic differences in the original ecosystem compared to the impaired, assemblages may struggle to thrive. Thus, restoration practitioners are faced with the joint challenges of restoring the degraded vegetation in the riparian zone and the biota that reside in the stream (Webb and Erskine, 2003).

With millions of dollars being spent annually on riparian and instream restoration projects to reverse the effects of channelization, practitioners are eager to find the best method to restore degraded habitats back to their natural state (Miller et al., 2010). As ecosystem engineers, beavers can naturally restore degraded streams by building dams that slow down water and increase the connectivity of the stream to its floodplain. Since introduction of beavers to most streams is not feasible, their use as a restoration tool is not a viable widespread solution. However, the installation of instream structures made from temporary sod plugs (TSPs) can mimic a natural beaver dam (Polluck et al., 2014). TSPs act like natural beaver dams by altering the flow of water by pushing water into the floodplains (Polluck et al., 2014). With the installation of these instream structures, practitioners have reported an increase in pool frequency, pool depth, and retention of woody debris and sediment (Roni et al., 2002). The increase in physical heterogeneity from the installation of TSPs results in a more diverse habitat for both macroinvertebrate and fish assemblages (Polluck et al., 2014).

To reverse the effects of chronic channelization within Deer Creek, the Denver Botanic Gardens implemented a riparian restoration project that consisted of installing TSPs and revegetating the riparian zone. In addition, water quality, vegetation, and macroinvertebrate data have been collected annually since 2016 as restoration endpoints. Whereas previous studies have focused primarily on the effects of the restoration project on riparian vegetation, I will describe how the macroinvertebrate assemblage has responded in the short-term to these restoration activities. Because certain macroinvertebrates are highly sensitive to hydrogeomorphic conditions that should improve over the three-year period of this study, the relative abundance of sensitive taxa would likely increase over the course of the restoration project. If this is true, I expect to see an increase in macroinvertebrate diversity at TSP sites over the three years and anticipate seeing that beaver and non-beaver sites would not change. In addition to seeing TSP sites improve, I expect to see diversity at its highest at beaver sites followed by TSP sites, then non-beaver sites.

## Methods

### Study site/ site selection.

The Denver Botanic Gardens at Chatfield Farms is located within the Chatfield Basin and is located in Jefferson County, Colorado (Figure 1). Chatfield Farms is home to over 550 plant species, 70 mammalian species, and hundreds of invertebrate species. The 700-acre Chatfield Farms is located approximately 15 miles south of the Denver metropolitan area and has a mosaic of plant communities comprising three main categories: agricultural pasture, grassland, and riparian. Deer Creek flows through Chatfield Farms and since the 1800s has been intensively managed resulting in a degraded riparian habitat. Management plans included channelization of the creek, which led to the loss of natural meandering flows (Figure 2).

In 2016, researchers from the Denver Botanic Gardens identified 12 transects along Deer Creek to conduct a restoration and monitoring study. At three of these transects, researchers installed instream TSP structures, replanted native vegetation, and removed non-native plants.

Three transects with beaver activity acted as positive controls while six transect without beaver activity acted as negative controls. In 2018, researchers added six more non-beaver transects as additional negative control sites for the restoration project. To evaluate whether macroinvertebrates can detect short term success in riparian zone restoration projects, I compared the macroinvertebrate assemblages across these 12 transects (TSP, beaver, non-beaver) within the Deer Creek watershed.

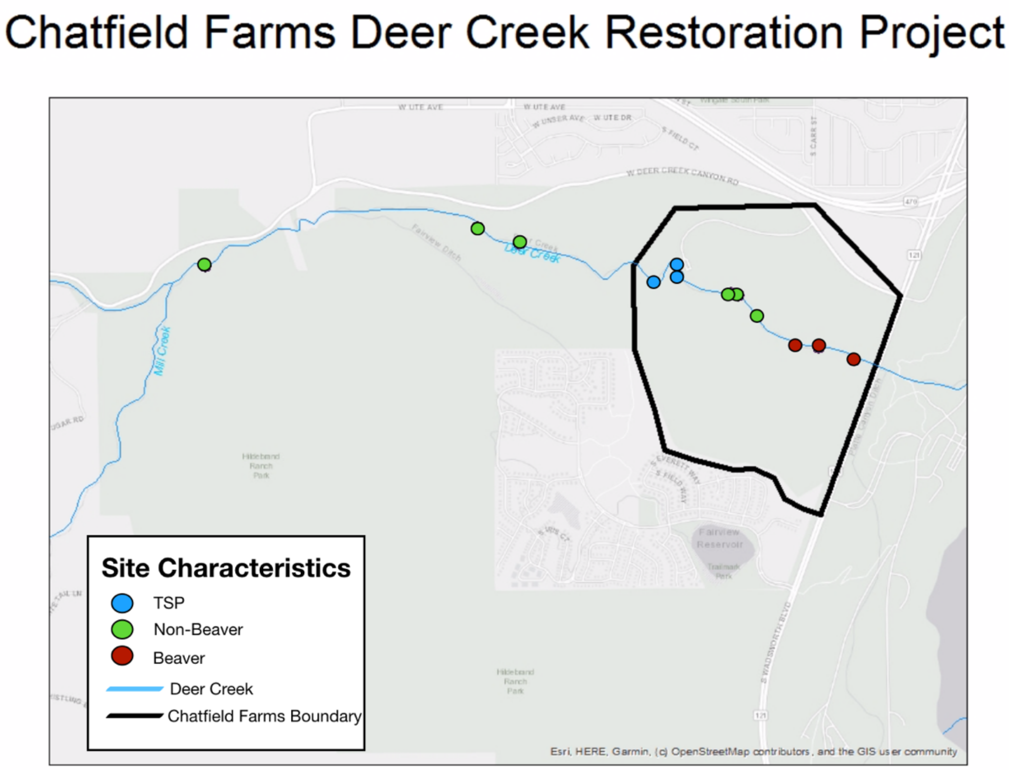


Figure 1: Site location map that represents the Chatfield Farms boundary line (black boundary line) with deer creek running through it (blue line) with each transect plotted. The blue dots with represent the TSP sites, the green dots represent non-beaver sites, and the red dots represent beaver sites. The black dots represent the sites that were added in 2018.



Figure 2: Site photographs of a channelized stream reach (A) and a reach that has natural beaver activity (B), both are photographs of Deer Creek at Chatfield Farms.

## Restoration Methods

### In-stream restoration.

Among the initial 12 transects researchers identified three restoration sites with proof of historical floodplains, and installed TSP structures in June 2016 (Figure 3). The goal behind installing TSPs was to raise water levels to stimulate overflow into historical floodplains. The TSP structures consisted of wooden stakes that held down biodegradable coconut fiber bags filled with organic fibers and/or gravel. In 2016, high flows breached the TSP structures, and in March 2017 these damaged TSPs were reinstalled and repaired.

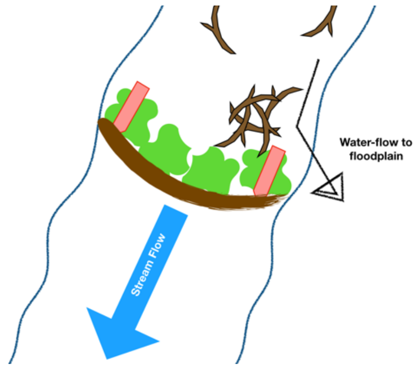


Figure 3: Diagram of TSP structures placed within restored sites to reconnect the stream to its floodplain by causing water to flow around the structure onto the historic floodplain.

**Vegetation restoration.**

In order to enhance native riparian vegetation, willow stakes and cottonwood plugs were planted along the stream bank and the floodplain at the three restoration sites. While researchers installed TSPs in 2016, they also removed understory vegetation near each TSP structure to allow waterflow onto the floodplain. The replanting of willows and cottonwoods across the sites aimed to attract native birds and wildlife into the habitat. In 2017, researchers continued with additional willow and cottonwood planting after TSP structures were repaired and reinstalled.

**Monitoring and transect description.**

DBG scientist measured physicochemical parameters and biological communities at each 25-m reach (12 in 2016 & 2017 and 18 in 2018) during June and July. From the original 12 reaches, three were defined as TSP sites, six were non-beaver sites, and three were beaver sites. At each transect stream characteristics including water appearance (clear, murky, foamy), macrohabitat proportions (riffle, pool, woody debris, undercut bank, runs), stream characteristics (thalweg, stream width), and water quality (temperature, pH, total dissolved solids, velocity, and dissolved oxygen) were recorded because of the influential role they play in stream water quality. Researchers also collected water samples to test for nutrients (nitrate and nitrite) and fecal coliforms including *Escherichia coli*.

For macroinvertebrate sampling, researchers distributed sampling locations proportionally among macrohabitats present (riffles, pools, runs). Sampling methods for macroinvertebrate collection included kick-netting and jabbing starting at the downstream end of the reach, using a D-frame net that is 500 μm mesh nylon. Macroinvertebrate samples from macrohabitats at each site were composited into one sample and transferred to bottles containing 70% ethanol then sent to GEI Consultants, Inc. for identification. The GEI laboratories enumerated macroinvertebrates to the lowest practical taxonomic level (typically species) and summarized the assemblage total taxon richness, EPT richness (the number of sensitive taxa, belonging to the mayfly (Ephemeroptera) stonefly (Plecoptera), and caddisfly (Trichoptera) orders), Shannon diversity index, EPT abundance, and Ephemeroptera abundance, all metrics that were scored and composited into the Colorado multimeric index (MMI).

**Analysis.**

To evaluate how macroinvertebrate assemblages differed across the TSP, beaver, and non-beaver site groups, I fit four generalized linear models that included site type, year, and their interaction (R Core Team, 2018). These models quantified how taxa richness, EPT abundance, Shannon diversity Index, and Colorado MMI differed among the 3 different transect types by year. Total taxon richness, and EPT richness were assumed to follow a Poisson distribution, whereas the Shannon diversity index and Colorado MMI were assumed to follow a normal distribution. After fitting these models, I conducted generalized linear hypothesis tests using the glht function in the multcomp package (Hothorn et al., 2008) to compare differences in each response among site types and year. I also used generalized linear hypothesis tests to calculate the average difference between beaver and TSP transects, beaver and non-beaver transects, and TSP and non-beaver transects across years. This allowed me to evaluate whether the macroinvertebrate biodiversity near TSP structures mimics those at natural beaver sites.

I also used a non-metric multidimensional scaling (NMDS) ordination on the Bray-Curtis distance matrix using the nmds function to depict the changes in macroinvertebrate community composition among the three different site types and the three different years. To assess whether community ordination scores correlated with physicochemical measurements I used the envfit function in the vegan package (Oksanen et al., 2019). Lastly, I used a permutational analysis of variance (permanova) on the Bray-Curtis distance matrix to statistically assess whether community structure varied by transect type (TSP, beaver, non-beaver), year, and their interaction.

**Results**

While average macroinvertebrate richness across years in sites with beavers was 26% (95% CI: 3 - 55%) higher than TSP sites, sites with beaver had 12% (95% CI: 3 – 25%) fewer taxa than sites without beaver (Figure 4). However, when considering richness of the sensitive EPT orders, beaver sites had 14% (95% CI: -17 – 57%) more EPT taxa than TSP sites across years, and .99% (95% CI: -1 – 1%) fewer than non-beaver sites. When comparing TSP and non-beaver sites for the average EPT richness, TSP sites had 53% (95% CI: 40 – 64%) fewer taxa than non-beaver sites (Figure 7). CO MMI scores at TSP sites did not significantly differ from sites with beaver ($p = 0.28$), they were -10 points lower (95% CI: -17 – -4, $p = 0.00116$) than sites without beaver (Figure 5). When observing diversity reported by the Shannon diversity index (H’) beaver sites had no significant difference between TSP or non-beaver sites (all p values > 0.124)

When calculating the annual trends at TSP sites, all metrics showed a significant decrease ($p < 1x10^{-07}$). EPT richness showed the most significant drop decreasing by 91% (95% CI: 79 – 96%) per year. For beaver sites CO MMI, taxa richness and EPT richness all decreased over the three years. EPT richness again showed the most significant decline decreasing by 87% (95% CI: 69 – 94%). On the other hand, diversity at beaver sites as reported by the Shannon diversity index (H’) significantly increased over the three years by 1.5 units per year (95% CI: 0.32 – 2.70, p = 0.00413). On average at non-beaver sites EPT richness, taxa richness, and CO MMI declined, whereas Shannon-Weaver Index (H’) increased by 1.08 units per year (95% CI: 0.23-1.92).

Over the last three years 148 macroinvertebrate taxa were recorded in the twelve sites at Deer Creek. The three most dominant taxa across all sites over the three years are Ephemeroptera, *Baetis tricaudatus*, diptera, *Dicrotendipes sp*., and the chironomid*,* *Phaenopsectra sp.* occurring at 21%, 16%, and 7%, respectively. A two-dimensional NMDS ordination (Figure 8, stress = 0.200) explained 70% (Axis 1 = 36%, Axis 2 = 34%) of variation in community structure across the twelve sites sampled from 2016-2018. A PERMANOVA indicated that macroinvertebrate community structure was not explained by site types ($R^2$= 0.03959, p = 0.87), year ($R^2$ = 0.022, p = 0.67), or their interaction ($R^2$ = 0.049, p = 0.67). Furthermore, no environmental variables (pH, temperature, total dissolved solids, E.coli, stream velocity, thalweg, dissolved oxygen) correlated significantly ($p < 0.05$) with macroinvertebrate community structure.

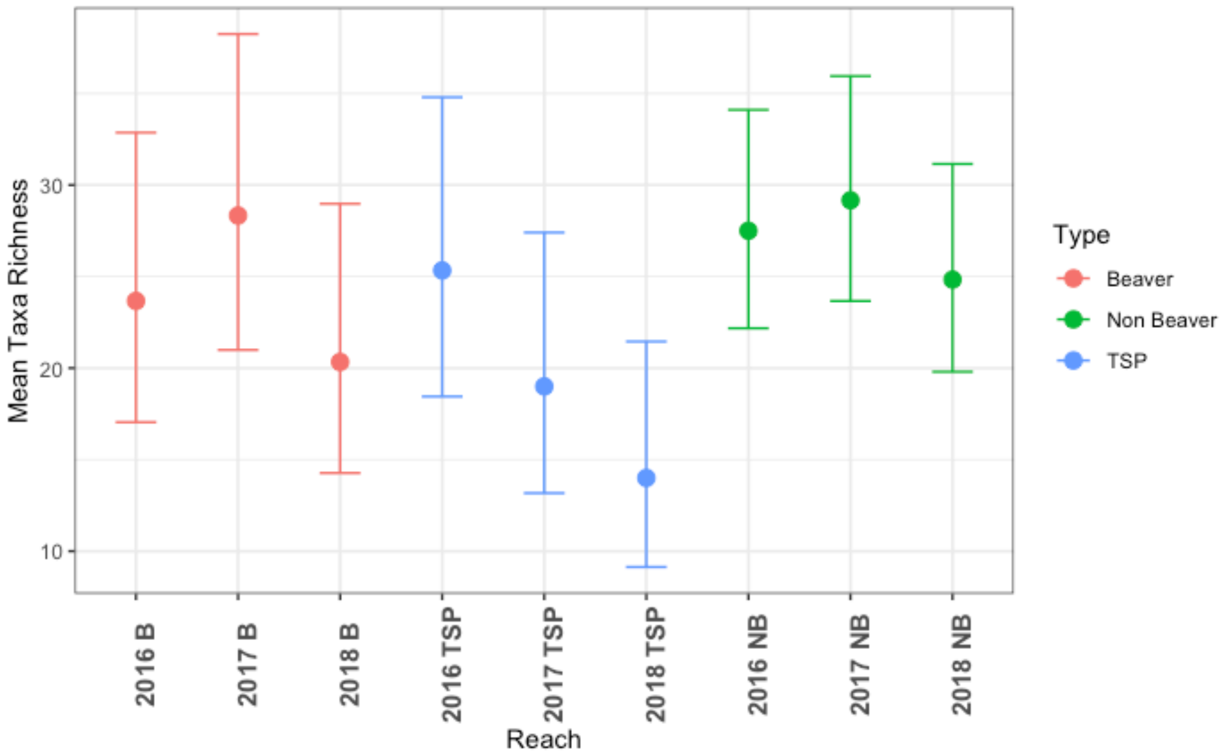


Figure 4: Beaver, TSP, and non-beaver sites showed a decrease in mean taxa richness over the three years. Beaver and non-beaver both increased in 2017 then decreased by 2018.

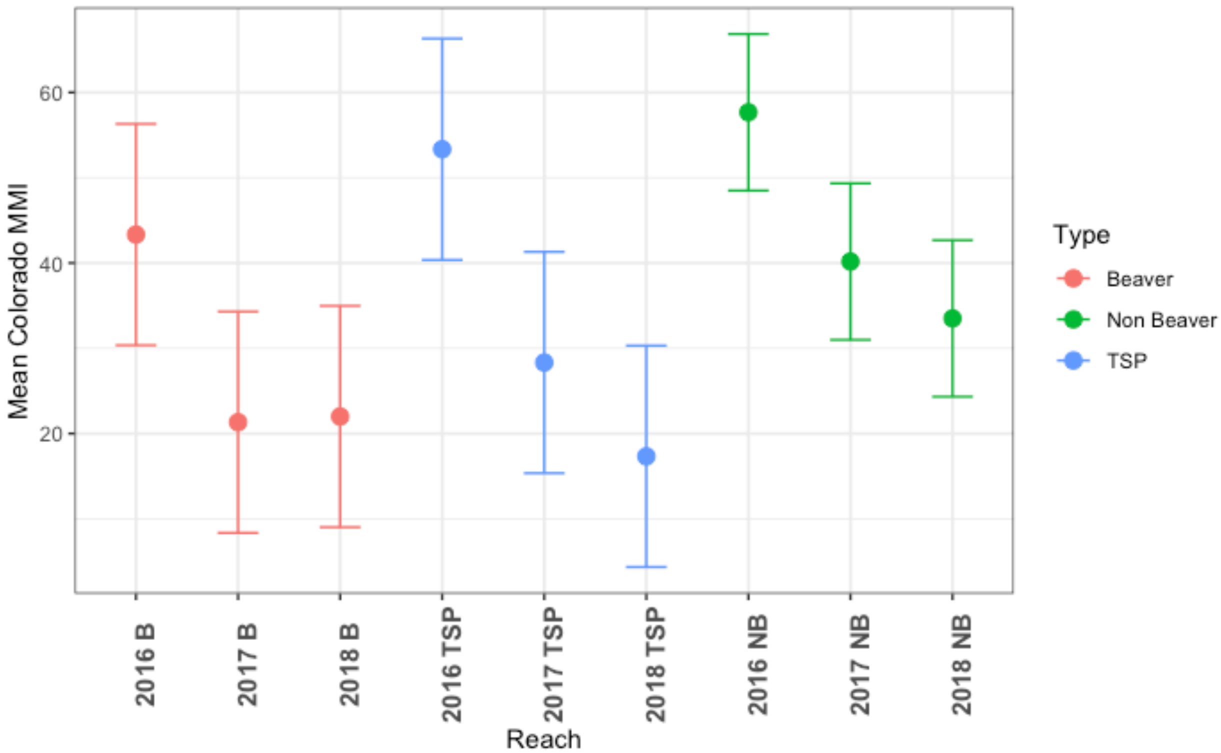


Figure 5: At all three sites beaver, TSP, and non-beaver the mean Colorado MMI score decreased each year significantly.

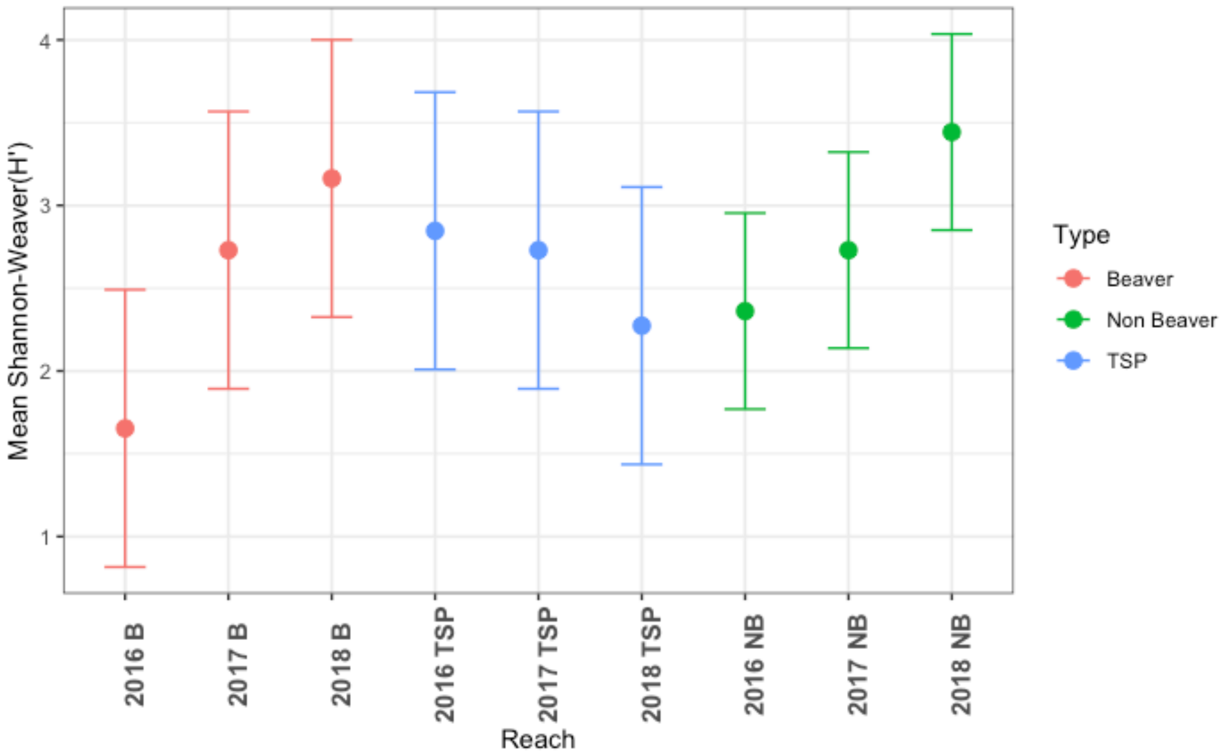


Figure 6: In response to Shannon-Weaver (H') both beaver and non-beaver increased each year, whereas TSP sites decreased.

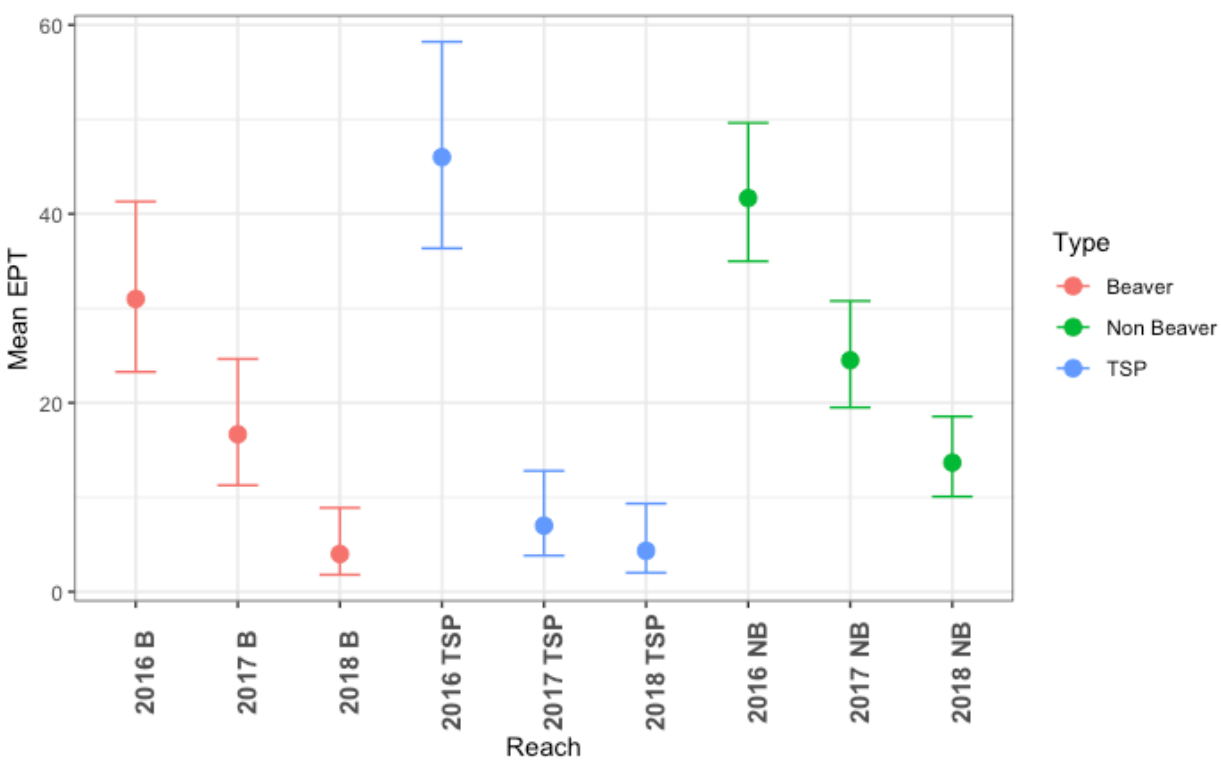

Figure 7: Across all sites mean EPT richness decreased significantly over time. The sensitive taxa within TSP sites decreased significantly between 2016 and 2017. Beaver and non-beaver sites had a more gradual decrease between each year.

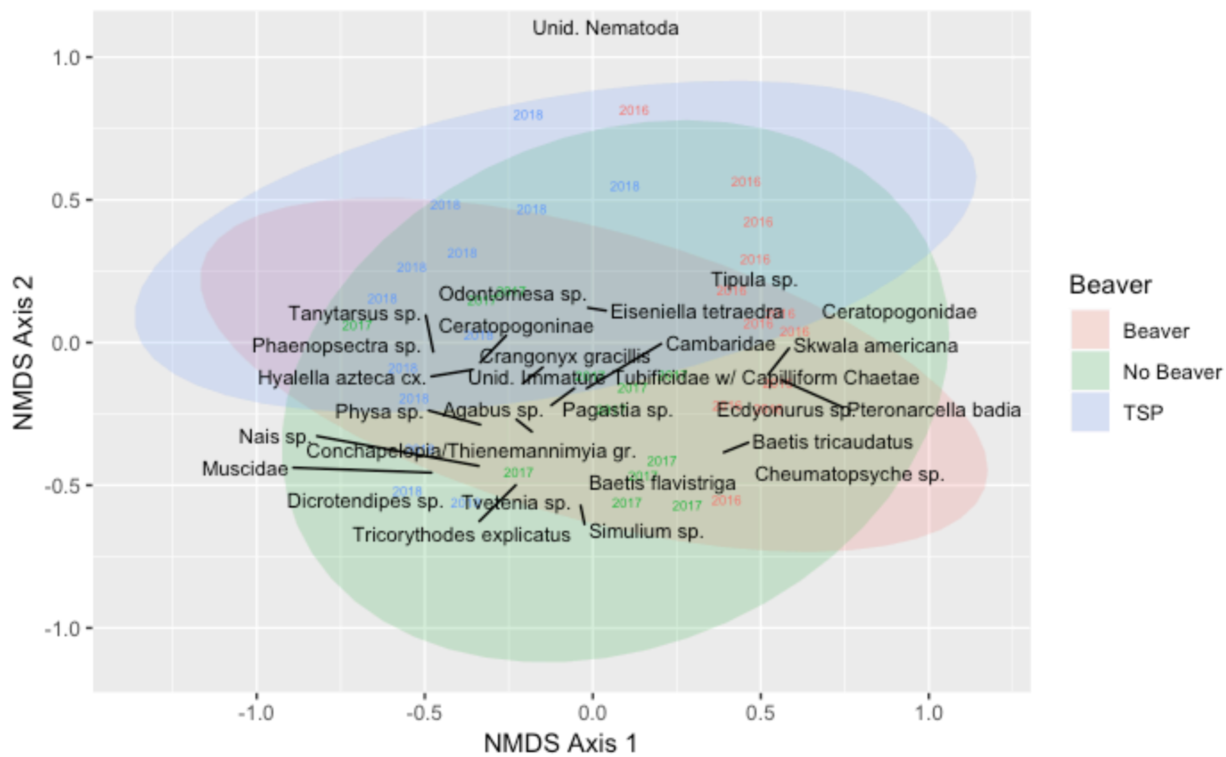


Figure 8: The NMDS ordination shows that non-beaver sites possess the majority of the taxa having a significant overlap with both TSP and beaver sites. The species within this ordination represents the top 20% most frequent species (29 species) within Deer Creek.

## Discussion

In this investigation of three years of post-restoration data in Deer Creek, CO I expected the macroinvertebrate biodiversity to improve over time at TSP sites because these sites begin to mimic sites with beaver. Contrary to my prediction, macroinvertebrate metrics decreased at across all sites as well over the three-year period. I also expected to see the highest macroinvertebrate diversity of macroinvertebrates at sites where beavers were present followed by where TSP were installed, because TSPs aim to mimic sites with beaver activity. Although according to this investigation beaver sites did not always withhold the highest macroinvertebrate diversity, because they do not retain a sufficient amount of water. Contrary to my predictions I found that sites without beaver had higher macroinvertebrate index scores than both beaver activity and TSP sites.

At restored sites where TSPs were installed macroinvertebrate metrics generally declined over time. The results from my study coincide with previous findings that show macroinvertebrate assemblages at restored sites decrease before they can increase (Laasonen, Muotka & Kivijärvi, 1998). The disturbance from installing the TSPs (hammering and laying of sod and rock bags) and re-installing them in 2017 could be the underlying factor for the trends we observed at TSP sites. Muotka and Laasonen (2002) also found that use of heavy machinery limits macroinvertebrate assemblages because it detaches benthic algae which macroinvertebrates need for food. These macroinvertebrates may take up to 10 years to recolonize and restablish after the heavy disturbance (Muotka and Laasonen, 2002; Muotka & Kivijärvi, 1998). Within Deer Creek, TSP instillation in 2016 and reinstallation in 2017 disrupt the stream bed by laying sod and rock bags and hammering stakes. Such disruption could have resulted in losses of macroinvertebrates at Deer Creek similar to those observed by Muotka and Lassonen (2002).

The results from this investigation not only showed a decline of macroinvertebrates in TSP sites, but also in reference (beaver) and non-reference (non-beaver) sites. Johnson et al. (2010) explained that when reference site quality declines it could be because reference sites were inadequately defined. The authors explain that reference sites have become more difficult to define because of the pressures from climate change (Johnson et al., 2010). The reference sites (beaver sites) used within this study may not in fact be true reference sites because of the larger scale pressures occurring at the watershed level. Within Deer Creek the amount of precipitation varied between the three years in which we collected data, which could be the reason the macroinvertebrate metrics also declined at other sites. Despite weather disturbance, larger scale watershed disturbances could have also ultimately impacted all sites. Although richness declined overtime at all sites, Shannon diversity increased at beaver and non-beaver sites. This counterintuitive increase in Shannon diversity may be due to the total abundance may be less spread across the taxa. This relates to a study done by Graça et al. (2004) who experienced an overall decline of macroinvertebrate abundance, thus increasing the evenness of a community.

Muotka et al. (2002) defined stream restoration as an unpredictable disturbance. Similarly, this study saw very little recovery in macroinvertebrates in a short period of time post-restoration. Muotka et al. (2002) suggest that long-term monitoring of macroinvertebrates and the riparian zone in restored and reference reaches is needed to assess whether restoration methods have enhanced the macroinvertebrate community or altered the existing community (Muotka et al., 2002). Nilsson et al. (2014) also conducted several follow-up studies to evaluate stream quality several years post- restoration. Their findings showed that even centuries post-restoration some streams never fully recovered. Harrison et al. (2004) suggest that some projects never restore because of the methods implemented during restoration completely eliminate biota. Since these previous studies advocate that recovery within a short period of time is unlikely, three years of post-restoration data may be insufficient for the DBG to observe an increase in macroinvertebrate diversity.

Over this time period I expected beaver sites to have the highest biodiversity, yet I did not see that, instead I saw that sites with no beaver activity actually withheld the greatest macroinvertebrate abundance. In this study, sites were defined as beaver, non-beaver, and TSP, although over the past three years some sites that are not considered "beaver" have shown recent beaver activity. One study acknowledges that the choice of reference sites is critical because these sites are the baseline for comparing non-reference sites (Sánchez-Montoya et al., 2009). Thus, defining a reference site as a non-reference site can result in an erroneous conclusion that the restoration was successful when it was not, or unsuccessful when it was. With beaver being our "reference site" having a clear understanding what makes up a beaver site is critical. Beaver activity throughout Deer Creek is constantly fluctuating, making it difficult to define where in the stream is considered "beaver".

Compounding the issue of accurate reference site identification and misunderstanding the scale at which a local restoration project might be the defining factor of why we did not see recovery. Palmer et al. (1997) suggested the "the field of dreams" hypothesis (i.e., if you build it, they will come), which implies that if a stream is restored locally then then biota will also recover. Previous literature concludes that in small scale restoration projects practitioners may not see a recovery due to large scale stressors. Purcell et al. (2002) evaluated restoration in a small urban stream called Baxter Creek in Northern California, and results showed that the restored creek quality has declined in comparison to the pre-restoration data. The authors suggest that restoring small scale urban streams can be difficult because water levels tend to be irregular throughout the dry summer season making it harder for biota to recolonize and reestablish (Purcell et al., 2002). In comparison to Baxter Creek, Deer Creek, Co has also experienced irregular water levels, in the year of 2018 five sites had no water resulting in no data collection. In conclusion, Purcell et al. (2002) suggests different restoration methods and monitoring techniques be used in small scale restoration projects.

One limitation of this study is that pre-restoration data on the macroinvertebrate assemblages was not collected. Approximately 10 % of restoration projects include post-project monitoring (Bernhardt et al., 2005). Not having pre-restoration data can make it difficult when monitoring changes over time. Pre-restoration data gives practitioners an idea of the condition of the stream prior to restoration. When restoration methods are executed practitioners then can compare their observations from pre-restoration to post- to accurately assess how the stream has changed. Miller et al. (2010) suggest that the lack of pre-restoration data can lead to an invalid assessment of the restoration project, because no baseline exists. In cases, for example, where macroinvertebrate diversity is naturally low post-monitoring assessment may enormously conclude that the methods executed to restore the stream failed, or the indicator species used to measure success was not ideal.

For future studies, the Denver Botanic Gardens should proceed with long-term monitoring of the abundance of macroinvertebrates to more accurately understand the extent of instream community recovery after this restoration project in Deer Creek. Future macroinvertebrate monitoring effort in deer creek may show recovery in later years. Furthermore, I only examined taxonomic diversity in this study. Rather than analyzing hundreds of diverse taxa, macroinvertebrates can be summarized by their functional feeding groups to report how functional measures such as energy processing may respond to stream restoration efforts.

In conclusion, my results showed that macroinvertebrate diversity did not recover at sites where TSPs were installed at Deer Creek. This finding may imply that the restoration methods or the indicator species (macroinvertebrates) may have not been the best fit for this restoration project. Furthermore, beaver sites which TSP sites were defined to mimic did not prove to have higher macroinvertebrate diversity. Therefore, suggesting that the sites defined beaver sites may have been inadequately defined.

**Literature Cited**


Bernhardt, E. S., & Palmer, M. A. (2007). Restoring streams in an urbanizing world. *Freshwater Biology*, *52*(4), 738-751.

Bernhardt, E. S., Palmer, M. A., Allan, J. D., Alexander, G., Barnas, K., Brooks, S., ... & Galat, D. (2005). Synthesizing US river restoration efforts.

Böhning-Gaese, K., & Bauer, H. G. (1996). Changes in species abundance, distribution, and diversity in a central European bird community. Conservation Biology, 10(1), 175-187.

de Waal, L. C., L. E. Child, P. M. Wade, and J. H. Brock, editors. 1994. Ecology and management of invasive riverside plants. John Wiley and Sons, New York.

Erwin, K. L. (2009). Wetlands and global climate change: the role of wetland restoration in a changing world. Wetlands Ecology and management, 17(1), 71.

Graça, M. A., Pinto, P., Cortes, R., Coimbra, N., Oliveira, S., Morais, M., ... & Malo, J. (2004). Factors affecting macroinvertebrate richness and diversity in Portuguese streams: a two scale analysis. International Review of Hydrobiology, 89(2), 151-164.

Herbst, D. B., & Kane, J. M. (2009). Responses of aquatic macroinvertebrates to stream channel reconstruction in a degraded rangeland creek in the Sierra Nevada. Ecological Restoration, 27(1), 76-88.

Hupp, C. R. (1992). Riparian vegetation recovery patterns following stream channelization: a geomorphic perspective. *Ecology*, *73*(4), 1209-1226.

Jähnig, S. C., Lorenz, A. W., Hering, D., Antons, C., Sundermann, A., Jedicke, E., & Haase, P. (2011).River restoration success: a question of perception. *Ecological Applications*, *21*(6), 2007-2015.

Jari Oksanen, F. Guillaume Blanchet, Michael Friendly, Roeland Kindt, Pierre Legendre, Dan McGlinn, Peter R. Minchin, R. B. O'Hara, Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens, Eduard Szoecs and Helene Wagner (2019). vegan: Community Ecology Package. R package version 2.5-4. https://CRAN.R-project.org/package=vegan

Johnson, R. K., Battarbee, R. W., Bennion, H., Hering, D., Soons, M. B., & Verhoeven, J. T. (2010). Climate change: defining reference conditions and restoring freshwater ecosystems. *Climate Change Impacts on Freshwater Ecosystems"(eds M Kernan, B Moss and RW Battarbee). Blackwell & Wiley*, 203-235.

Johnson RR, Haight LT. 1984. Riparian problems and initiatives in the Amer- ican Southwest: A regional perspective. Pages 404–412 in Warner RE, Hen- drix KM, eds. California Riparian Systems: Ecology, Conservation and Productive Management. Berkeley: University of Calfornia Press.

Jones, K. B., Slonecker, E. T., Nash, M. S., Neale, A. C., Wade, T. G., & Hamann, S. (2010). Riparian habitat changes across the continental United States (1972–2003) and potential implications for sustaining ecosystem services. *Landscape Ecology*, *25*(8), 1261-1275.

Laasonen, P., Muotka, T., & Kivijärvi, I. (1998). Recovery of macroinvertebrate communities from stream habitat restoration. Aquatic Conservation: Marine and Freshwater Ecosystems, 8(1), 101-113.

Miller, S. W., Budy, P., & Schmidt, J. C. (2010). Quantifying macroinvertebrate responses to in stream habitat restoration: applications of meta-analysis to river restoration. *Restoration Ecology*, *18*(1), 8-19.

Mlambo, M. C. (2014). Not all traits are 'functional': insights from taxonomy and biodiversity ecosystem functioning research. Biodiversity and Conservation, 23(3), 781-790.

Moore A.A. & Palmer M.A. (2005) Invertebrate biodi-versity in agricultural and urban headwater streams: implications for conservation and management. Ecological Applications, 15, 1169–1177.

Muotka, T., & Laasonen, P. (2002). Ecosystem recovery in restored headwater streams: the role of enhanced leaf retention. Journal of applied Ecology, 39(1), 145-156.

Muotka, T., & SYRJÄNEN, J. (2007). Changes in habitat structure, benthic invertebrate diversity, trout populations and ecosystem processes in restored forest streams: a boreal perspective. *Freshwater Biology*, *52*(4), 724-737.

Muotka, T., Paavola, R., Haapala, A., Novikmec, M., & Laasonen, P. (2002). Long-term recovery of stream habitat structure and benthic invertebrate communities from in-stream restoration. Biological conservation, 105(2), 243-253.

Naiman, R. J., & Decamps, H. (1997). The ecology of interfaces: riparian zones. *Annual review of Ecology and Systematics*, *28*(1), 621-658.

Nilsson, C., Polvi, L. E., Gardeström, J., Hasselquist, E. M., Lind, L., & Sarneel, J. M. (2015). Riparian and in-stream restoration of boreal streams and rivers: success or failure?. *Ecohydrology*, *8*(5), 753-764.

Pollock, M. M., Beechie, T. J., Wheaton, J. M., Jordan, C. E., Bouwes, N., Weber, N., & Volk, C. (2014). Using beaver dams to restore incised stream ecosystems. *Bioscience*, *64*(4), 279-290.

Purcell, A. H., Friedrich, C., & Resh, V. H. (2002). An assessment of a small urban stream restoration project in northern California. Restoration Ecology, 10(4), 685-694.

Purcell, A. H., Corbin, J. D., & Hans, K. E. (2007). Urban riparian restoration: an outdoor classroom for college and high school students collaborating in conservation. *Madroño*, 258-267.

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Resh, V. H. (2008). Which group is best? Attributes of different biological assemblages used in freshwater biomonitoring programs. *Environmental Monitoring and Assessment*, *138*(1 3), 131-138.

Resh, V. H., & Rosenberg, D. M. (1993). *Freshwater biomonitoring and benthic macroinvertebrates* (No. 504.4 FRE).

Richardson, D. M., Holmes, P. M., Esler, K. J., Galatowitsch, S. M., Stromberg, J. C., Kirkman, S. P., ... & Hobbs, R. J. (2007). Riparian vegetation: degradation, alien plant invasions, and restoration prospects. *Diversity and distributions*, *13*(1), 126-139.

Roni, P., Beechie, T. J., Bilby, R. E., Leonetti, F. E., Pollock, M. M., & Pess, G. R. (2002). A review of stream restoration techniques and a hierarchical strategy for prioritizing restoration in Pacific Northwest watersheds. *North American Journal of Fisheries Management*, *22*(1), 1-20.

Sánchez-Montoya, M. M., Vidal-Abarca, M. R., Puntí, T., Poquet, J. M., Prat, N., Rieradevall, M., ... & Álvarez, M. (2009). Defining criteria to select reference sites in Mediterranean streams. Hydrobiologia, 619(1), 39.

Torsten Hothorn, Frank Bretz and Peter Westfall (2008). Simultaneous Inference in General Parametric Models. Biometrical Journal 50(3), 346--363.

Webb, A. A., and W. D. Erskine. 2003. A practical scientific approach to riparian vegetation rehabilitation in Australia. Journal of Environ- mental Management 68:329–341.

# CHAPTER 4: ENVIRONMENTAL STAKEHOLDER ANALYSIS

The reintroduction of Gunnison prairie dogs to restore semiarid grasslands within the Rio Mora National Wildlife Refuge

## Introduction

When prairie dogs are present in grasslands, they improve groundwater recharge, regulate soil erosion, and increase carbon storage and forage availability (Martínez-Estévez, 2013). Several semiarid grasslands within North America, including those at the Rio Mora National Wildlife Refuge (RMNWR), were transformed to shrublands within the past 150 years (Bahre, 1995). At the RMNWR as the Gunnison prairie dog (GPD) population begin to decline the grasslands begin to degrade (USFWS, 2012). The degradation of these grasslands due to the woody encroachment led to isolated patches of grass ecosystem, resulting in very low diversity across the fragmented areas (Whitford, 1997). Although there are many methods to restore grasslands, reintroducing GPD has many beneficial outcomes besides restoring grasslands (e.g. aerating soil, providing habitat for other species). Reintroducing GPD within the RMNWR will help prevent future encroachment of woody shrubs, restore the ecosystem, and restore the GPD population that once thrived on the refuge. In order to control the new colony within one area away from where bison typically roam and to help prevent them from wondering to neighboring land, the GPD will be brought into upland grasslands that are not typically roamed by bison and pitfall traps will be created to prevent them from wandering.

## Summary of Environmental Issue

Occupying approximately 13% of the earth's surface and holding about 20% of the global carbon storage, grassland ecosystems are one of the world's most widespread terrestrial ecosystems (Scurlock and Hall, 1998). Since grasslands are one of the most common ecosystems understanding the effects of degradation is vital. As grasslands begin to decline, the carbon balance, the biodiversity within a habitat, and food production can all be negatively affected, causing a cascading affect within an ecosystem (Cai et al., 2015). Over the past 150 years, the rate of woody encroachment in grasslands within the southwestern United States increased dramatically (Buffington and Herbal, 1965). With the shift from grasses to dominant shrub species, alteration in vegetation cover may also cause changes in the soil chemistry (Bird et al., 2007). This shift in soil chemistry can ultimately affect the competitiveness between plant species, potentially providing more beneficial nutrients for woody shrubs (Torbert et al., 2012). As woody shrubs continue to invade grasslands, they not only effect the soil chemistry but also the soil. Respiration. Grasslands have 20% higher rates of soil respiration compared to habitats invaded by woody shrubs (Raich and Tufekciogul, 2000). Approximately 10% of atmospheric $CO_2$ passes through soils each year (Raich and Potter, 1995). Thus, when vegetation cover shifts from grassland to shrubland species, soil respiration will likely decrease. This decrease in soil respiration can negatively affect the global C cycle.

Within the RMNWR, the grasslands have begun to shift to have more abundant woody shrubs altering the overall biodiversity within the ecosystem. This increase in shrub density could be due to the decline of prairie dogs present on the refuge. When prairie dogs are present, they help maintain grasslands by preventing invasion and establishment of shrubs though their foraging and clipping (Davidson et al., 2018). Their constant clipping also promotes plant growth which contributes to the increase of plant biodiversity (Alliance, 2001). The burrows they dig will provide habitat for several species (e.g. birds, snakes, weasels), and the GPD themselves will become food for larger prey (e.g. coyotes, foxes, hawks; USFWS, 2015). Not only do their burrows provide habitat but as they dig, they aerate the soil causing the soil to be richer in nitrogen, phosphorous, and organic matter (Alliance, 2001). Possible solutions to prevent future woody encroachment on the refuge and to restore the ecosystem back to its native grassland include: ground level chaining, controlled burning, and reintroducing prairie dogs.

The first possible solution, ground level chaining, occurs when a chain is dragged across the ground to pull up woody shrubs. This process can improve seedbed conditions, which can accelerate the recruitment of new grass (Ansley, 2006). The second common method to reverse shrub encroachment is controlled burning. Fires are said to be most effective during the early stages of woody shrub encroachment, as they will use the fuel from herbaceous plants to burn through and destroy woody shrubs (Ansley, 2006). When the woody shrubs are dense, a burn may not be sustained because there is an insufficient amount of fuel from herbaceous plants (Wink and Wright, 1973). Lastly, reintroducing GPD can restore not only their declining populations but also the ecosystem functions they provide. Reintroducing prairie dogs at the Sevilleta National Wildlife Refuge (SNWR) in New Mexico restored both native grasses by clipping the roots of establishing shrubs, and animal biodiversity (e.g., lizards, snakes, arid, land birds) by providing habitat (Davidson et al. 2018; USFWS, 2012). SNWR a neighboring refuge to the RMNWR has also experienced woody encroachment and used prairie dogs to restore its grasslands. Thus, the RMNWR may witness similar success in using GPD to stem the effects of woody encroachment and restore their grassland ecosystem.

The GPD range is limited to high elevation (1830 – 3660 m) mountain valleys and plateaus in the southern Rocky Mountains (Finch, 1991), and they are distributed across the Four Corners region of the United States (Utah, Colorado, New Mexico and Arizona). The northernmost population of GPD is found in South Park, CO, while the southernmost population resides near the Mogollon Mountains in southwestern New Mexico (Pizzimenti and Hoffmann 1973). GPD are considered graminivores, they feed primarily on grasses, herbs and leaves (Shalaway and Slobodchikoff, 1988). Survivorship in the first year for prairie dogs is typically less than 60% (Hoogland, 2001). Major mortality factors are disease, predation, and humans. Their biggest predators are badgers, coyotes, and weasels (Martínez-Estévez 2013). Colonies suffer drastic population declines and are often extirpated during outbreaks of flea-borne sylvatic plague (Hoogland, 2001). Human impacts to the GPD population are usually caused by hunters. GPD are typically considered to be pests and are shot for invading agricultural land. Currently the GPD is listed as least concern, but their population has been decreasing since 1961 (USFWS, 2015).

**Stakeholders**

A proven method to help prevent further encroachment of woody shrubs and restore an ecosystem as a whole is by reintroducing GPD (Martínez-Estévez, 2013; Davidson et al., 2018). Stakeholders that are affected by the degradation of these grasslands are USFWS, birders, local tribes, ranchers, farmers, and surrounding refuges.

The USFWS has monitored how the decline of prairie dogs shifted grassland ecosystems to shrubs, therefore altering habitat availability for other animals, soil composition, and food production (USFWS, 2015; Davidson et al., 2012). Since the RMNWR is associated with the USFWS, they share a common goal not only to restore grasslands, but also to see GPD thrive within the refuge (USFWS, 2015). Thus, reintroducing GPD within the refuge will allow USFWS to meet the joint goals of restoring a grassland back to its historical condition and returning a native species to its ecosystem.

While the USFWS observed the degradation of grasslands, birders begun to see a shift in bird biodiversity. Over the last 50 years there has been a drastic decline of grassland and arid land birds within North America (Audubon, 2018). A reason behind this decline of bird species is the decline of grasslands themselves. Since birders concern on the bird's population in grasslands begin to grow, the Audubon New Mexico Conservation Ranching Program (ANMCRP) created an overarching goal to enhance biodiversity in grassland ecosystems. ANMCRP has monitored many grasslands within the New Mexico region where bird populations have decreased by nearly 80% (Audubon, 2018). When GPD are reintroduced, they will begin to create burrows which will provide habitat for grassland and arid land birds. Therefore, using GPD to restore the RMNWR grasslands will contribute to restoring the native bird biodiversity the birders have seen decline.

Similar to the Audubon society, Native American tribes appreciate the importance of conservation and value native species and ecosystem functions (Lamb et al., 2006). With an overarching goal to restore the native biodiversity within the refuge the RMNWR allowed the Pojoaque tribe to have a bison herd on the refuge. Having the bison on the refuge increased the native biodiversity in vegetation due to their grazing (RMNWR, 2015). Therefore, when the RMNWR grasslands begin to degrade the Pojoaque tribe became concerned since the herd relied on grazing on these grasslands (RMNWR, 2015). Reintroducing prairie dogs will help restore the grasslands to their historic condition and provide the proper vegetation for the Pojoaque tribe's bison herd. Although the reintroduction of GPD may put the bison herd at a higher risk of injuries due to prairie dog holes, but these burrows will also provide habitat for other species that not only the tribes wish to see thrive again but also the USFWS (RMNWR, 2015; USFWS 2015).

Since the encroachment of woody shrubs is an issue throughout the New Mexico region (Davidson et al., 2018), several neighboring refuges/land owners by the RMNWR are most likely also exhibiting encroachment. Having this degradation over a large region can negatively impact not only the biodiversity the tribe, USFWS, and birders wish to see restored but also, soil chemistry, and soil respiration (Cai et al., 2015; Bird et al., 2007). Subsequently, initiating a plan to restore the grasslands at the RMNWR will not only benefit the refuge but also surrounding refuges and landowners by providing them insight on a successful restoration plan.

Several stakeholders (e.g. hunters, the agricultural industry.) are negatively affected by the reintroduction of GPD although they still are not completely opposed to the idea. Ranchers are typically opposed to the idea of having prairie dogs on their land because of the danger their burrows put on cattle (Lamb et al., 2006). A review done by Reading et al. (2002) explained the overall dangers of having a prairie dog colony within the same land as their cattle, expressing that the burrows that prairie dogs make are very dangerous. These burrows have reportedly caused the cattle to break their legs, costing ranchers a mass amount of money on vet expenses (Reading et al., 2002). Despite the cons of having a prairie dog colony within the same area as cattle, they can also be very beneficial. As encroachment continues to increase, where the cattle roams to forage will soon be covered by woody shrubs. Controlling a small population of prairie dogs can help maintain the grassland and prevent further encroachment (Reading et al., 2002).

Similar to ranchers most farmers view prairie dogs as pests as they forage on their crops (Reading et al., 2002). A very small percentage of farmers do favor having a small colony of medium-sized prairie dogs on their land because of the benefits prairie dogs bring to soil composition (Lamb et al., 2006). Like farmers hunters have two views on prairie dogs, 1) they are aware that prairie dogs are keystone species for grassland ecosystems as a whole and respect the conservation of them, or 2) they are typically paid by land developers or farmers to shoot and eliminate the pest (Reading et al., 2002).

## Recommendation

Several stakeholders are affected by the decline in prairie dog abundance which has led to the encroachment of woody shrubs in grasslands. The ideal solution to restore these grasslands that benefits several stakeholders is reintroducing GPD. In order to successfully reintroduce the GPD, each stakeholder whether affected by the problem or by the potential solution should be taken into consideration to evaluate whether reintroducing GPD is the overall best method to restore the ecosystem. Stakeholders that are affected by the loss of grasslands are affected in different ways, whether it's a shift in soil composition, the loss of bird diversity, or the lack of grasses for herds to graze/forage on. Although some stakeholders are negatively impacted by the reintroduction of GPD, each stakeholder can benefit from the reintroduction of GPD.

In order to successfully reintroduce GPD and allow them to thrive within the refuge to restore the ecosystem back to its historic condition, three steps must be executed: 1) the initial reintroduction, 2) prevention of plague and disease, and 3) instituting regulations to end recreational shooting.

First, GPD will be collected from areas where their extermination is imminent (e.g. urban development site, agricultural land). Once the population is collected, the GPD will be tested for disease to determine if they are ready for reintroduction. After they are declared disease free, they will be introduced into the grassland ecosystem within the RMNWR. In order to keep track of the GPD community, species surveys on 500x500m plots will be conducted in order to determine the population size. To monitor for plague or disease, any deceased prairie dog found will be assessed to determine if the cause of death was sylvatic plague (Arizona Game and Fish Department, 2007). In addition to determining the cause of death, each deceased prairie dog will also be bagged and disposed of properly to help prevent disease transmission. Since sylvatic plague can affect other species (e.g. deer, mice), stakeholders will be informed if the GPD colony has been affected. Lastly, in order to minimize the amount of shooting of GPD, hunters will be informed about the decline of prairie dogs and how that effects the ecosystem as a whole. GPD will then be taken off the small game list for public lands which will prevent shooting from being a common issue. This will also help secure the new population and allow the refuge to monitor the population as a whole.

With these three steps to reintroduce GPD within the RMNWR, the RMNWR will begin to see the ecosystem transforms back grasslands. As the GPD begin to thrive within the refuge USFWS, birders, and the RMNWR staff will begin to see their joint goal of having biodiversity restored take place. Despite some of the negative impacts GPD bring (e.g. destruction of crops, danger to herds) to stakeholders they can also be very beneficial. For farmers, GPD are known to aerate the soil which allows crops the thrive (Bird et al., 2007). Similar to farmers, ranchers also have a difficult time accepting the benefits of GPD due to the constant danger their burrows put on cattle, although a small population of prairie dogs can help with maintain a healthy grassland for cattle to graze on (Lamb et al., 2006). In order to prevent GPD from constantly crossing over to farmer/ranchers' lands, pitfall traps can be installed in order to prevent GPD from crossing certain boundaries (Dickman et al., 1995). This instillation of pitfall traps will not only allow the RMNWR to maintain their colony, but also provides stakeholders with the satisfaction that the RMNWR is staff is working to prevent their colony from invading other lands. In relation to ranchers, the Pojoaque tribe may voice concern about the safety of their bison herd. In order to ensure the safety of the bison herd the new prairie dog colony will be maintained in upland grassland areas that the bison do not frequently roam, again using pitfall traps to help prevent them from wondering out of specific areas (Dickman et al., 1995). This will allow the Pojoaque tribe to see that their bison herd is not only safe but also watch GPD restore the historic land. Stakeholders, whether they are the farmers, ranchers, Pojoaque tribe, USFWS, or the birders will be able to see the transformation within the ecosystem as a whole and will then be able to fully appreciate the important role GPD play within their ecosystem.

**Literature Cited**


Alliance, P. C. (2001). Restoring the prairie dog ecosystem of the Great Plains: Learning from the past to ensure the prairie dog's future. Predator Conservation Alliance, Bozeman, Montana.

Ansley, R. J., Wiedemann, H. T., Castellano, M. J., & Slosser, J. E. (2006). Herbaceous restoration of juniper dominated grasslands with chaining and fire. *Rangeland Ecology & Management*, *59*(2), 171-178.

Audubon. (2018, April 24). Grassland Conservation. Retrieved from http://nm.audubon.org/news/grassland-conservation

Bird, S. B., Herrick, J. E., Wander, M. M., & Murray, L. (2007). Multi-scale variability in soil aggregate stability: implications for understanding and predicting semi-arid grassland degradation. *Geoderma*, *140*(1-2), 106-118.

Buffington, L.C., Herbel, C.H., 1965. Vegetation changes on a semidesert grassland range from 1958 to 1963. Ecol. Monogr. 35, 139–164.

Cai, H., Yang, X., & Xu, X. (2015). Human-induced grassland degradation/restoration in the central Tibetan Plateau: The effects of ecological protection and restoration projects. *Ecological Engineering*, *83*, 112-119.

Davidson, A. D., Detling, J. K., & Brown, J. H. (2012). Ecological roles and conservation challenges of social, burrowing, herbivorous mammals in the world's grasslands. Frontiers in Ecology and the Environment, 10(9), 477-486.

Davidson, A. D., Hunter, E. A., Erz, J., Lightfoot, D. C., McCarthy, A. M., Mueller, J. K., & Shoemaker, K. T. (2018). Reintroducing a keystone burrowing rodent to restore an arid North American grassland: challenges and successes.

Dickman, C. R., Predavec, M., & Downey, F. J. (1995). Long-range movements of small mammals in arid Australia: implications for land management. Journal of Arid Environments, 31(4), 441-452.

Finch, D. M. (1991). Threatened, endangered, and vulnerable species of terrestrial vertebrates in the Rocky Mountain Region. *Gen. Tech. Rep. RM-215. Fort Collins, CO: US Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. 38 p.*, *215*.

Hoogland, J. L. (2001). Black-tailed, Gunnison's, and Utah prairie dogs reproduce slowly. *Journal of Mammalogy*, *82*(4), 917-927.

Lamb, B. L., Reading, R. P., & Andelt, W. F. (2006). Attitudes and perceptions about prairie dogs. In Conservation of the black-tailed prairie dog: Saving North America's western grasslands (pp. 108-114). Island Press Washington, DC, USA.

Martínez-Estévez L, Balvanera P, Pacheco J, Ceballos G (2013) Prairie Dog Decline Reduces the Supply of Ecosystem Services and Leads to Desertification of Semiarid Grasslands. PLoS ONE 8(10): e75229. https://doi.org/10.1371/journal.pone.0075229

Pizzimenti, J. J. and R. S. Hoffmann (1973). *Mammalian Species:* Cynomys gunnisoni. The American Society of Mammalogists, 1.

Raich JW & Potter CS (1995) Global patterns of carbon dioxide emissions from soils. Global Biogeochem. Cycles 9: 23–36

Raich, J. W., & Tufekciogul, A. (2000). Vegetation and soil respiration: correlations and controls. Biogeochemistry, 48(1), 71-90.

Reading, R. P., Clark, T. W., McCain, L., & Miller, B. J. (2002). Black-tailed prairie dog conservation: A new approach for a 21st century challenge. *Endangered Species Update*, *19*(4), 162-170.

Rio Mora National Wildlife Refuge [RMNWR]. (2015). Pueblo of Pojoaque Bison. Retrieved from https://windriverranch.org/pueblo-of-pojoaque-bison

Scurlock, J.M.O., Hall, D.O., 1998. The global carbon sink: a grassland perspective. Glob. Change Biol. 4, 229–233.

Shalaway, S., & Slobodchikoff, C. N. (1988). Seasonal changes in the diet of Gunnison's prairie dog. *Journal of Mammalogy*, *69*(4), 835-841.

Torbert, H. A., Polley, H. W., & Johnson, H. B. (2012). Nitrogen and Carbon Cycling in a Grassland Community Ecosystem as Affected by Elevated Atmospheric CO2. International Journal of Agronomy, 2012.

USFWS. (2012, September 24). Wildlife & Habitat - Sevilleta - U.S. Fish and Wildlife Service. Retrieved from https://www.fws.gov/refuge/Sevilleta/wildlife_and_habitat.html

USFWS. (2012). Land Protection Plan. *Rio Mora National Wildlife Refuge and Conservation Area.*

USFWS. (2015). Gunnison's prairie dog. Retrieved from https://www.fws.gov/mountainprairie/es/gunnisonPrairieDog.php

Whitford, W. G. (1997). Desertification and animal biodiversity in the desert grasslands of North America. *Journal of Arid Environments*, *37*(4), 709-720.

Wink, R. L., and H. A. Wright. 1973. Effects of fire on an Ashe juniper community. Journal of Range Management 26:326–329.