



Understanding the influence of climate, genetic variation, and herbivory on aspen (*Populus tremuloides*) growth in Yellowstone National Park

Elaine M. Brice and Daniel MacNulty*

Department of Wildland Resources, Utah State University, Logan, UT

*Author for correspondence: dan.macnulty@usu.edu

Abstract Predation is commonly cited as a top-down effect that structures food webs, with the reintroduction of wolves to Yellowstone as perhaps the most famous example. However, despite two decades of research, there is still debate as to whether wolves (*Canis lupus*) have indirectly benefited aspen (*Populus tremuloides*) by reducing herbivory from elk (*Cervus canadensis*). As such, the purpose of this study was to investigate the role of top-down and bottom-up forces on aspen recruitment in northern Yellowstone. The UW-NPS grant funds were used to conduct a genetic analysis of 59 aspen stands in an effort to determine whether genetic variation is one control of aspen recruitment. During summer 2018, 122 leaves were collected and sent to the Mock Lab at Utah State University. The samples will undergo DNA extractions at 12 microsatellite loci, which will provide us with genotype and ploidy level, with the genotype at a resolution to distinguish clones. Concurrently, we measured aspen heights and browse rates, as well as soil moisture. The median aspen height in 2018 was 110 cm (SD=135), and the browse rate was 0.45. Stands varied in volumetric water content (VWC), ranging from a mean VWC of 2.7% to 45.2%.

Introduction

Understanding the forces that drive food web structure can be challenging in terrestrial, free-living systems (Ford and Goheen, 2015), and scientists have long debated the strength of top-down and bottom-up processes (Dobson, 2014). Predation is commonly cited as a top-down effect that structures food webs across a range of systems and taxa (Winnie and Creel, 2017). Most studies on top-down effects, however, occur in aquatic systems with small, short-lived predators, and the effects of large terrestrial carnivores are, therefore, less understood (Ford and Goheen, 2015; Schmitz et al., 2004, 2000). Several studies have reported that large terrestrial predators can promote vegetation growth by negatively interacting with herbivore species (Ray et al., 2013, 2005; Terborgh and Estes, 2010). However, due to the com-

plex nature of studying free-living systems, such research is often observational, thereby lacking replication and control (Ford and Goheen, 2015).

Perhaps the most famous example of predation as a top-down effect is that of wolf reintroduction in Yellowstone National Park (YNP). Wolves (*Canis lupus*) were eradicated from YNP in the 1920s, removing the main predator of elk (*Cervus canadensis*) from this system (Smith et al., 2016). As a result, the elk population increased substantially, and researchers observed a decrease in plant biodiversity. Wolves were then reintroduced in 1995, and researchers began documenting decreases in elk abundance and increases in the growth of deciduous woody vegetation shortly thereafter (Ripple et al., 2001). Aspen (*Populus tremuloides*) is one species that has received a great deal of attention since wolf reintro-

duction, with observations of increased height and decreased browsing rates in northern YNP (Beschta et al., 2018). Aspen provides up to 60% of elk diet in winter (Eisenberg et al., 2013), and researchers have subsequently proposed that the observed recovery is due to decreased elk herbivory as a result of wolf predation (e.g., Beschta et al., 2018; Laundré et al., 2001). An alternative hypothesis is that bottom-up processes, such as drought and site factors, are more important drivers of aspen recruitment (e.g., Kauffman et al., 2010; Vucetich et al., 2005). Largely absent from the discussion on aspen recovery is the potential role of genetic variation, which has not been tested as a driver of aspen recruitment in northern YNP. Aspen genetics are important to consider, as certain genotypes can be more resilient to drought and herbivory (Mock et al., 2012). With many potential drivers of aspen recruitment, there is no scientific consensus on the mechanisms causing change in YNP.

The aim of this project is to determine the indirect impact of wolves on aspen by quantifying the effect of herbivory, water availability, and genetic variation on aspen recruitment in northern YNP over the last two decades. By determining the relative importance of herbivory versus these other factors, we can infer the strength of the relationship between wolves and aspen. This relationship is critical to understanding the extent to which wolves are responsible for the ecological changes in YNP that have occurred since their reintroduction. The overarching question we are attempting to answer is: *What is the indirect impact of wolves on aspen recruitment?* To answer this question, we will examine the relative roles of herbivory, water availability, and genetic variation on aspen growth at 113 transects across northern YNP (Figure 1). The UW-NPS grant is funding the genetic component of our research question, which has not been previously tested but could prove highly informative. For this aspect of the project, we aim to determine the following:

1. Are our aspen stands all genetically distinct?
2. How does genotype vary among stands, and does this account for variation in recruitment across the northern range?

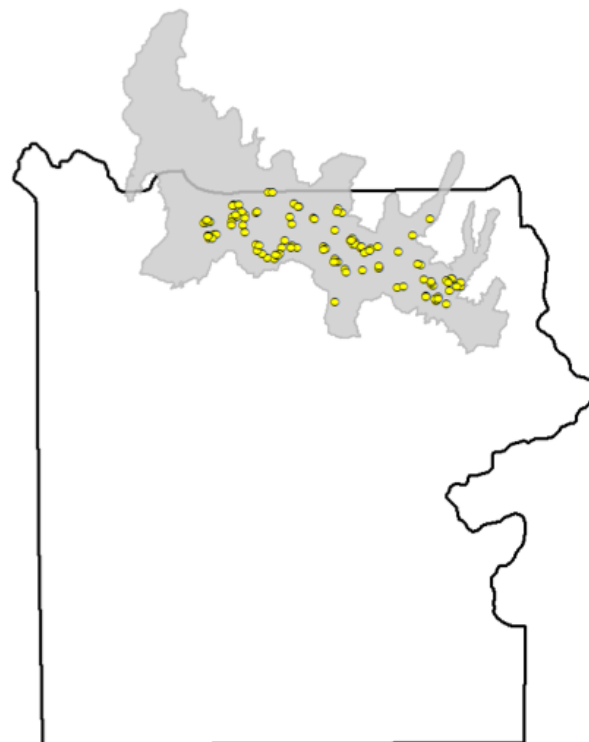


Figure 1. Map of the study area. Yellowstone National Park is outlined in black, with the northern range in grey. Yellow points are our 113 aspen transects.

Aspen in the Rocky Mountain region is primarily clonal, reproducing through root suckering that creates large stands of genetically identical stems (Barnes and Han, 1993). Such clonal aspen stands can cover many hectares (Mock et al., 2012), making it difficult to determine distinct individuals by sight alone. The majority of research on aspen in northern YNP assumes that stands separated by 30 meters are separate clones (e.g., Beschta et al., 2016, 2018; Painter et al., 2014, 2015; Ripple and Beschta, 2007, 2012). However, this assumption has not been tested, and could lead to erroneous conclusions regarding the health of each stand. As such, our first goal was to determine whether our aspen stands are all genetically distinct. In contrast, it is possible that some stands have more genetic individuals than others. The 1988 fires that burned over 700,000 ha in YNP (Kay, 1993) triggered seedling establishment across the park, thereby introducing genetic variation (Romme et al., 2005), with most of this varia-

tion within aspen stands rather than between stands (Stevens et al., 1999). Another purpose of this study, therefore, is to determine if aspen stands in YNP have differing amounts of genetic variation, and whether this variation would lead to different responses to herbivory and drought.

Our second aim was to determine if genotype varies by aspen stand, as genotype could predict the resilience of aspen to browsing and environmental change. Aspen is generally diploid or triploid, meaning it has either two or three copies of each chromosome. Triploid clones have reduced fertility, but are large and long-lived. Additionally, they are often found in drought-prone regions, and are proposed to be more successful in harsh environments (Mock et al., 2012). However, only 3% of sampled clones in the Greater Yellowstone Ecosystem (GYE) are triploid, with 97% as diploid (Mock et al., 2012). If the ratio of diploid to triploid stands in northern YNP differs from the GYE, it could perhaps explain some of the variation in aspen recruitment, as some stands may be more browse- or drought-resistant than others. Furthermore, the presence of triploidy could signify which stands are likely to survive the increased drought conditions that are predicted with climate change.

Our research will quantify the link between aspen and wolves, which has only been hinted at in the literature, and further our understanding of the role of large, mammalian predators in structuring food webs. The UW-NPS grant is aiding in this research by allowing us to address the role of bottom-up processes, such as genetic variation, which will enhance our ability to tease out the effect of herbivory, and, therefore, wolves.

Methods

Dr. Eric Larsen (Univ. Wisconsin – Stevens Point) and YNP staff have measured aspen at 113 20x1 meter transects across northern YNP since 1999 (Figure 1). Long-term measurements included: (1) counting the number of young aspen along each transect, (2) measuring height and current annual growth, and (3) determining if individuals have been browsed. With the

assistance of a field technician, we returned to each transect during the summer of 2018 to continue collecting data. In addition to the three measurements outlined above, we also deployed soil moisture sensors, tested surface soil moisture with an instant-read probe, and collected leaf tissue samples for genetic analysis. Six soil moisture sensors were placed at two sites with high mean transect heights, two sites with moderate mean transect heights, and two sites with low mean transect heights. The sensors were buried in the middle of the transect 15-cm deep in the soil, and set to measure the soil water content every two hours. The sensors will operate for one year, and we will return to export the data in the summer of 2019.

The instant-read probe was used to measure the volumetric water content (VWC) of the first 12-cm of the soil at 62 sites. We measured the VWC every 4-m along the transect, resulting in six measures per transect (0m, 4m, 8m, 12m, 16m, 20m). We then calculated the mean and standard deviation of the VWC at each transect to determine how much soil moisture varies within a stand.

Finally, we collected leaf tissue samples for genetic analysis at 59 sites (Figure 2). A single leaf was collected from one young aspen every 5m along the transect, with each leaf having a surface area slightly larger than a quarter. If there was no aspen at the meter-mark, we collected a leaf from the closest aspen within 2m. If there were no aspen along the transect, we collected a leaf from an individual within the stand, unless no aspen were present. Each leaf was placed in an individual paper coin envelope and stored in an open container of silica gel so as to properly dry. After data collection, leaf samples were sent to Karen Mock's genetic analysis lab at Utah State University to be processed. Here, the samples will undergo DNA extractions at 12 microsatellite loci. These loci will provide us with the genotype and ploidy level, with the genotype at a resolution to allow us to distinguish clones. The \$5,000 grant will support the processing of 90-120 samples; as such, we sent in a subset of 1-3 leaves from each transect to be analyzed, for a total of 122 samples from 59 plots. Due to the uncertainty in how many samples can be processed, the lab will first analyze one sample taken

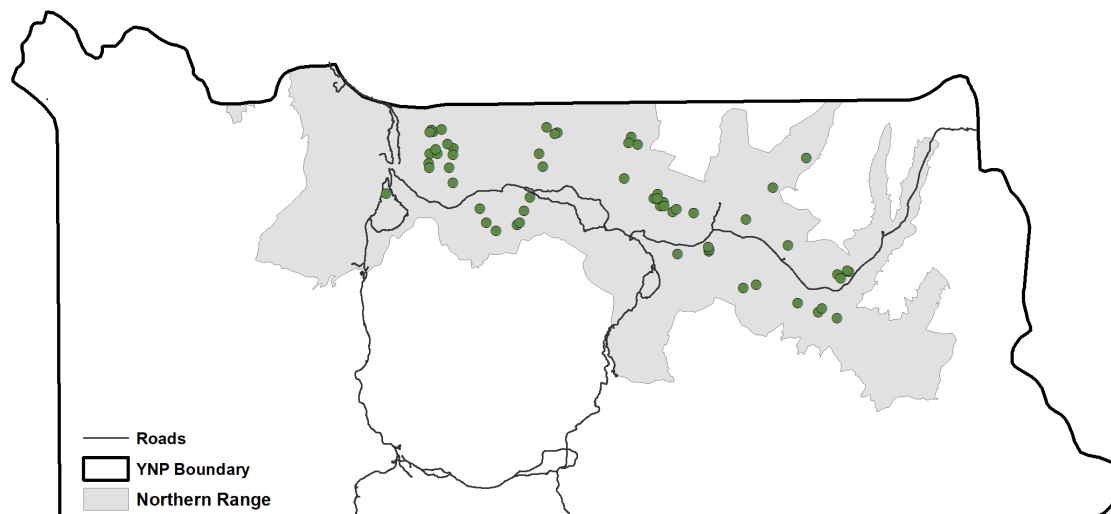


Figure 2. Subset of aspen stands where leaves were collected for genetic analysis ($N_{\text{stands}} = 59$).

from the start of each of the 59 transects to establish whether these stands are distinct clones. Then, the lab will analyze a second leaf sample from the end of the transect for 46 of the 59 sites. If the two samples from a single site are the same individual, the transect as a whole is likely a single individual, as we are comparing aspen that are 20-m away. Finally, if any funds remain, the lab will analyze a third sample from the 10-m mark for 7 of the 59 sites. The results will be returned to us in January 2019.

Once the leaf samples have been processed, we will build hierarchical Bayesian models in R to determine the extent to which genetic and site factors account for variability in aspen height and browse. Hierarchical models account for the spatial and temporal autocorrelation of our aspen dataset, and Bayesian methods allow us to include prior information regarding our parameters. Aspen height and browse will be our dependent variables, with herbivory, genotype, and water availability as our independent variables.

Preliminary Results

Data from the genetic analysis will be completed in January 2019; as such, we cannot speak to the outcome from this aspect of our project. However, we can report the status of aspen height growth and browse rates, as well as a preliminary investigation

into soil moisture. First, the median height of aspen across northern YNP in 2018 was roughly equivalent to that of 2016 and 2017, at 110 cm compared to 113 and 100 cm for 2016 and 2017, respectively (Figure 3a). The standard deviation (SD) increased slightly to 135 cm, as opposed to 125 and 126 for 2016 and 2017, respectively (Figure 1). While aspen heights in 2018 were similar to the most recent years, there was an increase of 70 cm in median height and 111 cm in SD since 1999. The proportion of aspen browsed was 0.45 in 2018, compared to 0.41 in 2017, and 0.47 in 2016 (Figure 3b). This is a decrease of 0.27 from the 1999 browse rate.

Soil moisture in aspen stands varied from a maximum mean VWC of 45.2% to a minimum mean VWC of 2.7% (Figure 4). The standard deviation varied from a maximum of 22.1% to a minimum of 0.57%. The majority (95%) of stands sampled had a mean VWC below 20%, and 92% had a SD less than 10%.

Conclusions

It is too early as of yet to make any conclusions regarding the effect of genetic variation on aspen recruitment in northern YNP. However, from the height data, it is evident that variation in aspen height growth has increased greatly since 1999, while the median height has increased less so, and appears to

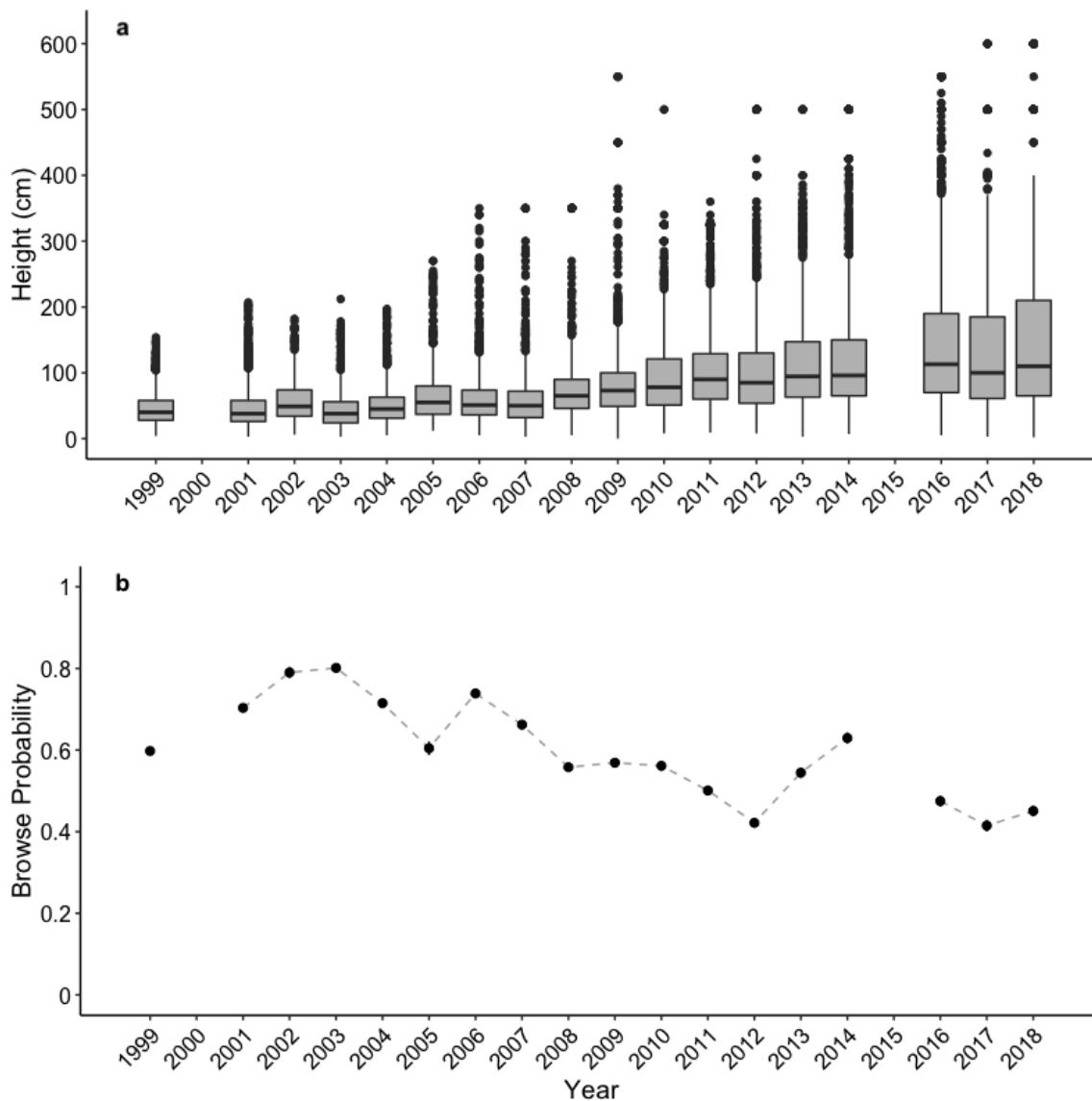


Figure 3. (a) Boxplots of young aspen heights at 113 stands in northern YNP from 1999 to 2018. Thick black lines denote the median height, boxes represent upper and lower quartiles, and points are outliers. (b) Proportion of young aspen browsed at 113 stands in northern YNP from 1999 to 2018. The points are obscuring the standard error bars for each year. Stands were not sampled in 2000 or 2015.

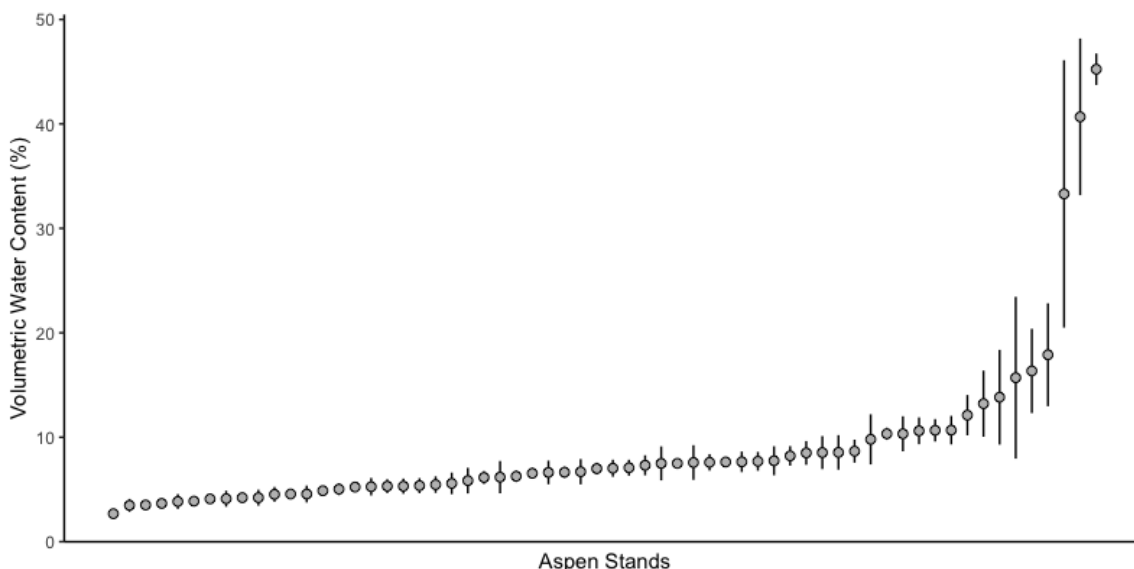


Figure 4. The percent volumetric water content (VWC) in each of 62 sampled aspen stands. Points represent the mean VWC of 6 measures along each transect, and the bars represent one standard error. Stands are ordered by mean VWC.

be reaching an asymptote. Alongside a decrease in browse rate, these results suggest that, as herbivory declined, other environmental regulators of aspen recruitment have taken effect. That is, herbivory was the limiting factor for recruitment when elk density was high, and now that herbivory has decreased, site factors have become limiting. Additionally, the soil moisture data suggests that stands do, indeed, differ in VWC, but that the majority are between 2.7% and 20%. Furthermore, it appears on the whole as though there is little variation in soil moisture within stands.

Future Work

The question of how wolves indirectly affect aspen in northern YNP is a complex one, and we therefore have many avenues to explore in the future. While it appears that site factors may be more important now that herbivory has decreased, we are still unsure of what those factors are. Future work needs to further expand and explore the genetic analysis of YNP aspen in relation to aspen recruitment and herbivory. Additionally, *in situ* soil moisture data needs to be collected so as to accurately compare the water availability in different stands. Another avenue for exploration is that of secondary compounds in the stems and bark of aspen, which could deter herbivory. Fi-

nally, these site factors need to be modeled in conjunction with herbivory in order to understand the amount of variation in aspen recruitment explained by each.

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