

We have been working to understand how hydrology, climate, and a restructured food web influence willow communities in the riparian ecosystems of the northern range of Yellowstone National Park. Our work during 2001 to 2010 provided a core dataset required for our successful LTREB proposal in 2012. The central goal of our LTREB is to determine if the grassland state that replaced willow (*Salix spp.*) communities after the extirpation of wolves in the early 1900's is stable despite wolf reintroduction or, alternatively, if reduced herbivory following the restructuring of the food web will allow willow communities and disturbance by beaver to reorganize to their historic conditions. We seek to understand the forces that stabilize or destabilize the alternative state. Our decadal research plan focusses on four questions:

1. Are willows on historic floodplains locked into a slow growing state as a result of changes in hydrology caused by loss of disturbance by beaver or are they approaching change-points in height and productivity?
2. How do climate and hydrology modify the effects of a restructured food web to explain spatial and temporal variation in willow growth?
3. Why has browsing pressure remained largely unchanged despite the addition of wolves to the food web and their top-down effects on elk numbers?
4. Will a disturbance regime restored by the return of dam building by beaver persist and expand or will the effects of intense herbivory by multiple species of herbivores cause dams to be abandoned by beaver, creating transient dynamics in disturbance?

*** What was accomplished under these goals (you must provide information for at least one of the 4 categories below)?**

Specific objectives

1. Add year nineteen to the time series of observations on willow height, net primary production, browsing intensity, and environmental covariates controlling plant growth using published protocols.
2. Continue observations of willow height, net primary production, browsing intensity and water table depth at sites occupied by beaver.

3. Build Bayesian models needed to support Results and Discussion in an Ecological Monograph reporting the outcomes of our LTREB funding.

Major Activities:

We collected new data on *Salix* spp. growing season productivity, height accumulation, and over-winter browsing intensity at our four experimental sites and 23 observational sites during spring and summer 2019. We collected seasonal data on hydrologic variation in the water table and stream depth. We monitored and downloaded data from environmental sensors at each treatment of our four experimental sites and eleven of our observational sites. The sensors record air temperature, precipitation, and soil temperature and moisture at 20 and 50 cm depths at one hour intervals. To further our understanding of plant-water relations, we surveyed tagged willows to quantify plant height above water table and distance to stream. We will incorporate these data into our Bayesian state-space model to assess the effects of climate on willow growth and response to browsing and hydrology.

Our experiment tested the hypothesis that the reintroduction of wolves cause a rapid change in riparian communities by releasing the dominant shrub, *Salix* spp., hereafter willows, from effects of browsing. The alternative hypothesis is that willows cannot grow tall without disturbance created by beaver dams, and that eliminating browsing alone will fail to allow height recovery. The experiment was a randomized complete block with a two by two factorial layout and for replications. A dam treatment (dammed and undammed) was established in 2001 to raise water tables, mimicking the effects of beaver dams. A browsing removal treatment (fenced vs unfenced) was established to mimic the purported effects of wolves on elk browsing. All cells in the treatment should behave similarly if wolves have released willows. Alternatively, cells in the dammed treatments should grow more rapidly if wolves have not released willows.

Total ungulate biomass has remained approximately constant on the northern range following reintroduction of wolves because bison numbers have increased coincident with declines in elk numbers. This means we would predict that total consumption of willow biomass has remained effectively constant after the reintroduction of wolves if bison consume willows, even relatively small amounts of willows. This prediction is consistent with our earlier observations of nearly constant browsing intensity since 2002. Indirect trophic effects of wolves on willows will be strongly

attenuated if bison are browsing willow to any significant extent, because wolves rarely prey on bison. We continued our work on using DNA in saliva residues on browsed stems collected during January-March, 2014 to determine which ungulate species were consuming willows. We collected samples along our experimental stream reaches to determine if elk, bison, moose, and mule deer preferentially select shoots when browsing.

Our sequencing of the mitochondrial genome has provided us with roughly 50 potential SNP markers that in different combinations can be used to indicate species. We have already chosen five that, when combined, provide accurate species identification. Our analyses are all run on a liquid based micro-array (Fluidigm biomark), a system that has proven very powerful for amplifying poor quality DNA samples. However, because we amplify the DNA saliva samples from twigs up to 6 months after deposition in the wild, some samples hold minimal quantities of DNA, occasionally causing PCR error. To minimize this risk our final panel will hold more markers to create marker redundancy for species assignment.

We collected samples of browsed willow tissue during May 2108. We believed these samples would be representative of late winter and early spring browsing because our extraction method has been shown to be able to successfully amplify DNA that has been exposed to the environment for more than 12 weeks, though the success rate of PCR amplifications deteriorates over time (Nichols et al., 2012). Six sites in riparian willow habitats were sampled. Two common species of willow were chosen, *Salix geyeriana* and *S. bebbiana*, both of which could be identified in the field. Willows shrubs were randomly selected from the sites, the number of willows sampled depended on willow abundance, ranging from three to four shrubs of each species per site (except at one site which had seen a decrease in willow abundance and only 1 willow of each species was available to sample). The samples were sequenced in the SciLifeLab in Stockholm, Sweden. Final results await bioinformatic analysis, which we anticipate will be completed during spring 2020.

Specific Objectives: Significant Results: Key outcomes or Other achievements:

We are completing analyses of all responses through 2019 including browsing intensity, above ground net primary production, and height growth. We will emphasize analysis of height responses here because these analyses are the most complete. Experimental treatments include two manipulations established in 2001: fences to prevent browsing by large herbivores and simulated beaver dams

to raise water tables. Treatments were arranged in a randomized complete block design with a factorial layout and four replications. We used a fully Bayesian hierarchical model to analyze these data (see attached model_description.pdf). We hypothesized that dams and fences would have no effect on willow growth if 1) hydrologic conditions following the loss of dam building by beaver do not limit willow growth and 2) wolves protect willows from browsing by large herbivores, releasing suppression of growth by herbivory. Accepting these hypothesis provides evidence for the operation of a trophic cascade (Beschta and Ripple, 2007, 2010; Ripple and Beschta, 2004a; Ripple et al., 2014). Rejecting them provides evidence that a trophic cascade is weak or entirely absent.

We observed dramatic effects of treatment on heights of willows at the end of the growing season (Figure 1). Willows in undammed, unfenced plots (hereafter, controls) grew slowly, averaging 3.56 cm per year (highest posterior density Bayesian credible interval, BCI = 2.84, 4.34). Dams more than doubled the growth rate in plots that were not fenced relative to controls (additive increase in growth rate relative to controls, hereafter effect size = 4.23, BCI = 3.08, 5.27) and fences in plots that were not dammed more than doubled the growth rate relative to controls (effect size = 5.11, BCI = 3.99, 6.21). Dams and fences together more than tripled the growth rate (effect size = 12.8, BCI = 11.4, 14.3). We observed an interaction between dams and fences. The combined treatment increased growth rate 3.49 cm/year (BCI = 1.49, 5.4) over the sum of the fence and dam effect.

[Figure 1 about here.]

We observed similar effects of treatment on spring heights. The effect size of fences was some smaller (effect size = 3.66, BCI = 2.94, 4.41) and the effects of dams somewhat greater (effect size = 6.05, BCI = 5.11, 6.91) than we observed for fall heights. The dam effect indicates that willows can withstand relatively heavy browsing as long as they are growing on shallow water tables. The dependence of the effect of fences on the effect of dams was substantially greater for spring heights than for fall heights (interaction = 4.39, BCI = 2.9, 5.92. This interaction indicates that the effect of reducing browsing are amplified when willows grow on sites with shallow water tables.

[Figure 2 about here.]

It is possible that the dramatic results observed in our experiment resulted because the experimental controls (undammed, unfenced) plots were anomalous and failed to represent other sites on

the norther range. We tested this idea by comparing heights of willows in the experimental controls with height of willows on 23 observational sites randomly chosen across the winter range in 2008. Measurements on the observational sites were identical to those on the experimental controls.

We observed no difference in growth rates of willows on experimental controls (mean growth rate = 4.27cm/year, BCI = 3.23, 5.23) relative to willows on observational sites (mean growth rate = 4.19cm/year, BCI = 3.42, 4.96) (Figure 3)

[Figure 3 about here.]

[Figure 4 about here.]

Additional evidence that our experiment formed a representative model for sites on the northern range is provided by comparison of fall heights of willows in the experimental controls, observational sites, dammed, unfenced experimental sites, and beaver occupied sites. There was no difference in heights between the experimental controls and the 23 observational sites indicating that our controls were representative of the larger population from which they were drawn. The dammed, unfenced plots were designed to mimic sites that could potentially be occupied by beaver. Heights of willows in beaver occupied sites closely resembled heights in the dammed, unfenced experimental plots.

[Figure 5 about here.]

Statistical distributions of height simultaneously reflect changes in the median height and changes in the variance of height. There was a strong upward trend in the variance of the distribution of fall heights throughout the study (Figure 6).

[Figure 6 about here.]

We focus here on three years spanning the time series to examine the change the shape of height distributions. Height distributions of willow on the northern range shifted rightward, toward taller values during 2001 - 2019, reflecting increases in mean height, while the width of these distributions expanded dramatically, reflecting increasing variance in height (Figure 7). A substantial proportion of individuals remained below 200 cm in height, a widely agreed upon threshold for restoration (Keigley and Frisina, 1998; Beschta and Ripple, 2007; Marshall et al., 2013) 7: (% of willow in

experimental controls were below the threshold height, BCI = %, % and % of willow in observational plots were below the threshold, BCI = %, % (Figure 7

[Figure 7 about here.]

The distribution of height of willows in experimental and observational datasets during 2008 strongly resembled the distribution of heights in a data set obtained in 1990 (Singer et al., 1994), five years before wolves were reintroduced (Figure 7 C), suggesting no distinguishable change in willow heights occurred during the thirteen years following wolf reintroduction. The height distributions of willows during 2019 shifted rightward relative to the 1990 distribution, but substantial overlap remained. Some, but not all, of the sites in the Singer dataset were the same as in our studies, so comparisons between these datasets are imperfect. However, the Singer data offer the only existing measurements of heights of willow before wolves were introduced and, hence, provide a valuable point of reference.

Two, broad generalizations emerge from this analysis. The average height of willows clearly increased following the reintroduction of wolves, the recovery of other members of the predatory guild, and increased harvest outside the park boundaries, all of which contributed to a marked decline in elk numbers. However, the change in median height was not nearly so large as the change in the variance in height. How should this be interpreted? This analysis suggests that perturbation of the food web by increases in wildlife and human predation caused declines in elk numbers. Herbivory was the primary control on height growth of willow and aspen before these declines occurred. Afterward, however, many ecosystem controls on plant growth could be expressed because herbivory was no longer dominant.

Notable among these controls are limitations in availability of resources for plant growth (Tercek et al., 2010). A substantial body of experimental evidence on willow growth on the northern range corroborates this idea – willows require adequate moisture to grow tall (Bilyeu et al., 2008; Marshall et al., 2013). The interaction between the fence effect and the dam effect reported here means that that the magnitude of the effect of removing herbivory depended on the effect of water availability (Figure 1). Ameliorating herbivory can enhance growth rate in plants with adequate water, removing herbivory can *retard* growth of plants that are moisture stressed (Johnston et al., 2007). Twenty five percent of variation in height of browsed willows was explained by spatial

variation in access to ground water determined by stable isotopes (Johnston et al., 2011).

The second generalization is this. The popular media and some scientific papers have promoted the idea that wolves caused a uniform, rapid, and dramatic shift in woody plant communities on the northern range. Our analysis of height distributions over time contradict this idea. Instead we have observed slow changes in plant height (Figure 7) that are highly variable over space. It is true that there are now tall willows the northern range (Figure 7 **A** - **B**), but it is also true that there were tall willows before wolves were reintroduced (Figure 7 **C**)

The analysis here shows why several studies have reached different conclusions, notably (Ripple and Beschta, 2004b, 2007; Beschta and Ripple, 2007, 2010; Ripple and Beschta, 2012). All of these studies made inference from a sampling design that focussed on the tallest plants in stands. When the variance of the distribution in height increases over time, then the tails of the distribution, including the tails containing observations of the tallest plants, become increasingly poor indicators of the mean response. Moreover, random sampling, or a formal procedure for adjusting inference from samples that are non-random, (e.g., Gelman et al., 2013, Chapter 8) is required to characterize the shape and centrality of all statistical distributions. Sampling the tallest plants in a stand rather than sampling randomly produces data incapable of accurately characterizing range-wide trends in plant communities.

The primary conclusion of this analysis is to change the way we think about trophic cascades in Yellowstone. Did restoration of predator guild and increased human harvest influence reduce elk numbers? This is virtually certain. Did reduction in elk numbers, in turn, cause a rapid and uniform increase in heights of willow and aspen? No. Instead, it appears that reduction in elk populations allowed herbivory to emerge as one of many controls limiting plant growth, controls that operate differently across the northern range.

Our experiment allows us to unequivocally reject the hypothesis that the reintroduction of wolves to the landscapes of the northern range of Yellowstone caused rapid restoration of willow communities. More than three decades have passed since wolves were restored. Most willows experiencing ambient conditions remain well below the 200 cm heights widely considered to be a threshold for restoration (Keigley and Frisina, 1998; Beschta and Ripple, 2007; Marshall et al., 2013) (Figures 1, 2). Most willows in treated plots exceed the restoration threshold in 2019.

These results provide evidence that the northern range exists in an alternative state stabilized

by the hydrologic changes caused by the loss of dam building by beaver that occurred in the mid-twentieth century after wolves were extirpated (Wolf et al., 2007). These changes dramatically compressed willow habitat, virtually eliminated willow establishment, and created moisture-stressed conditions for willows that were established (Wolf et al., 2007). Removing browsing *amplified* water stress by changing the morphology of willow to growth forms with diminished water use efficiency (Johnston et al., 2007). Our experimental and observational plots provide a clear, mechanistic explanation of the consequences of these changes: water stressed plants cannot respond rapidly to the ecosystem perturbation caused by the restoration of an apex predator. Predator-induced reductions in herbivory may be necessary for restoration of riparian plant communities, but they are not sufficient.

*** What opportunities for training and professional development has the project provided?**

A post-doc, a masters student, and two undergraduates were supported by these awards.

*** How have the results been disseminated to communities of interest?**

*** What do you plan to do during the next reporting period to accomplish the goals?**

We will continue to collect data on willow growth and utilization and hydrologic variation throughout the growing season. Julia Jansson, a PhD student with Goran Spong at the Swedish University of Agricultural Sciences, is now conducting genetic analysis needed to identify the species of browsers consuming willows on beaver occupied and unoccupied sites on the northern range. Hobbs is leading development of a Bayesian state-space model to explain how ungulate numbers and patterns of predation risk explain spatiotemporal variation in browsing intensity and willow growth. The project will use this model to determine how trophic effects are modified by climate and hydrology. We will continue our studies of effects of browsing and water availability on population processes using a capture-mark-recapture analysis of decadal patterns of survival of individually marked stems.

1 Products

Books

Conference Papers and Presentations

Inventions

Journals

Licenses

Other Products

Hobbs gave an extensive interview to the Denver Channel television station about the implications of our work in Yellowstone for a Colorado ballot initiative requiring that wolves be reintroduced to the state. See <https://www.thedenverchannel.com/news/360/supporters-farmers-at-odds-over-reintroducing-wolves-to-colorado>.

Other Publications

Rolf O. Peterson, Robert L. Beschta, David J. Cooper, N. Thompson Hobbs, Danielle Bilyeu Johnston, Daniel Kotter, Eric J. Larsen, Daniel R. MacNulty, Kristin N. Marshall, Luke E. Painter, William J. Ripple, Douglas W. Smith and Evan C. Wolf. In press. Indirect effects of carnivore restoration on vegetation. Pages ____ in *Yellowstone Wolves*. University of Chicago Press.

This book chapter represents an unusual achievement in two ways. First, the authors include all of the graduate students who have worked on this project since its inception. It is perhaps of greater significance that the chapter includes authors of decidedly disparate views on the evidence support trophic cascades in Yellowstone, Robert Beschta, William Ripple, Tom Hobbs, and David Cooper. The chapter provides the first synthetic effort aimed at resolving these different views.

Patents

Technologies or Techniques

Thesis/Dissertations

Websites

2 Changes/Problems

Changes in approach and reason for change

Actual or Anticipated problems or delays and actions or plans to resolve them

Changes that have a significant impact on expenditures

We plan our final field season during 2019. The absence of a graduate student means that post-docs and the PI's will do all data analysis and preparation of manuscripts for peer-reviewed journals. This will require shifting funds originally allocated to the graduate student to the PI's and a post-doc.

significant changes in use or care of human subjects

significant changes in use or care of vertebrate animals

significant changes in use or care of biohazards

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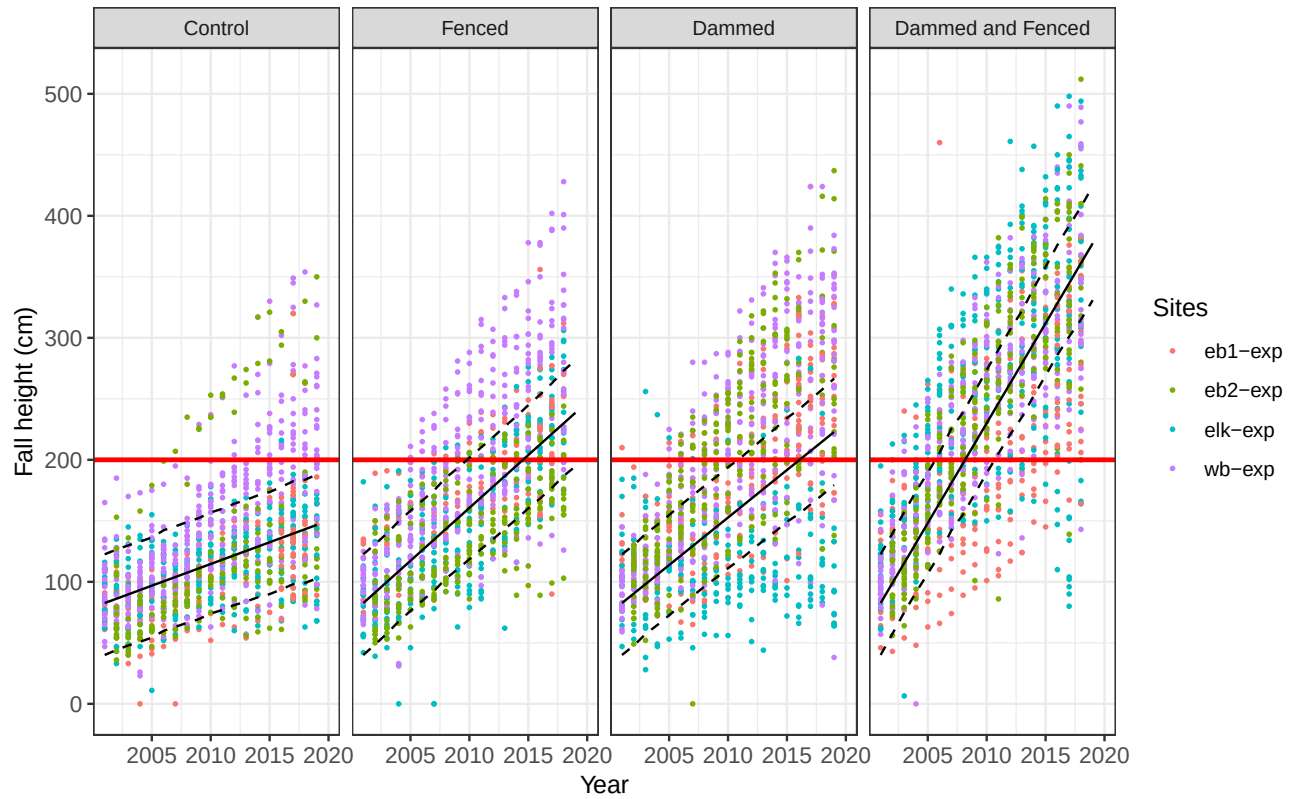


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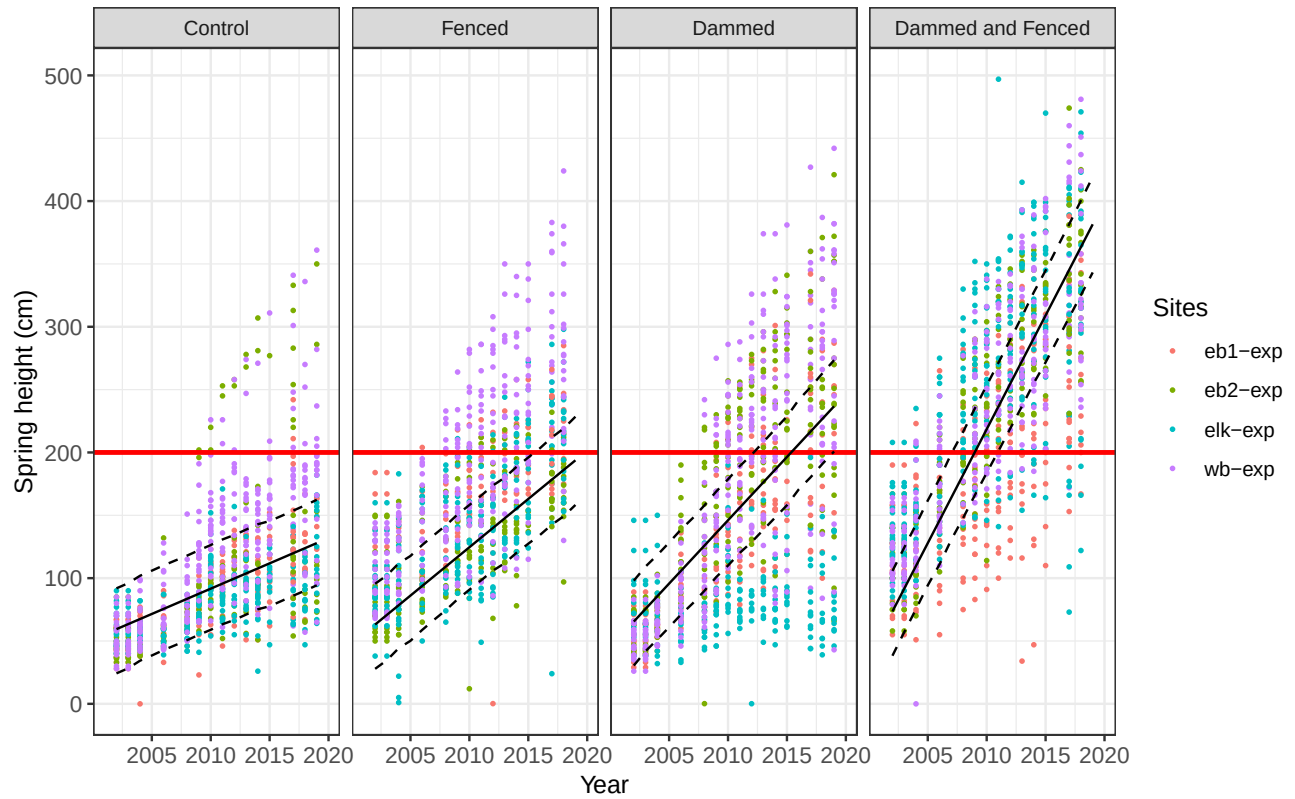


Figure 2: Observations of heights of willows at the beginning of the growing season during 2002 - 2019 in response to treatment at four replicated sites. Solid lines are medians of the posterior predictions of fall height at a new site; dashed lines show 95% highest posterior density intervals. The horizontal dashed red line represents the threshold for restoration, the height at which seed production is assured because some stems are too tall to be browsed by elk and bison.

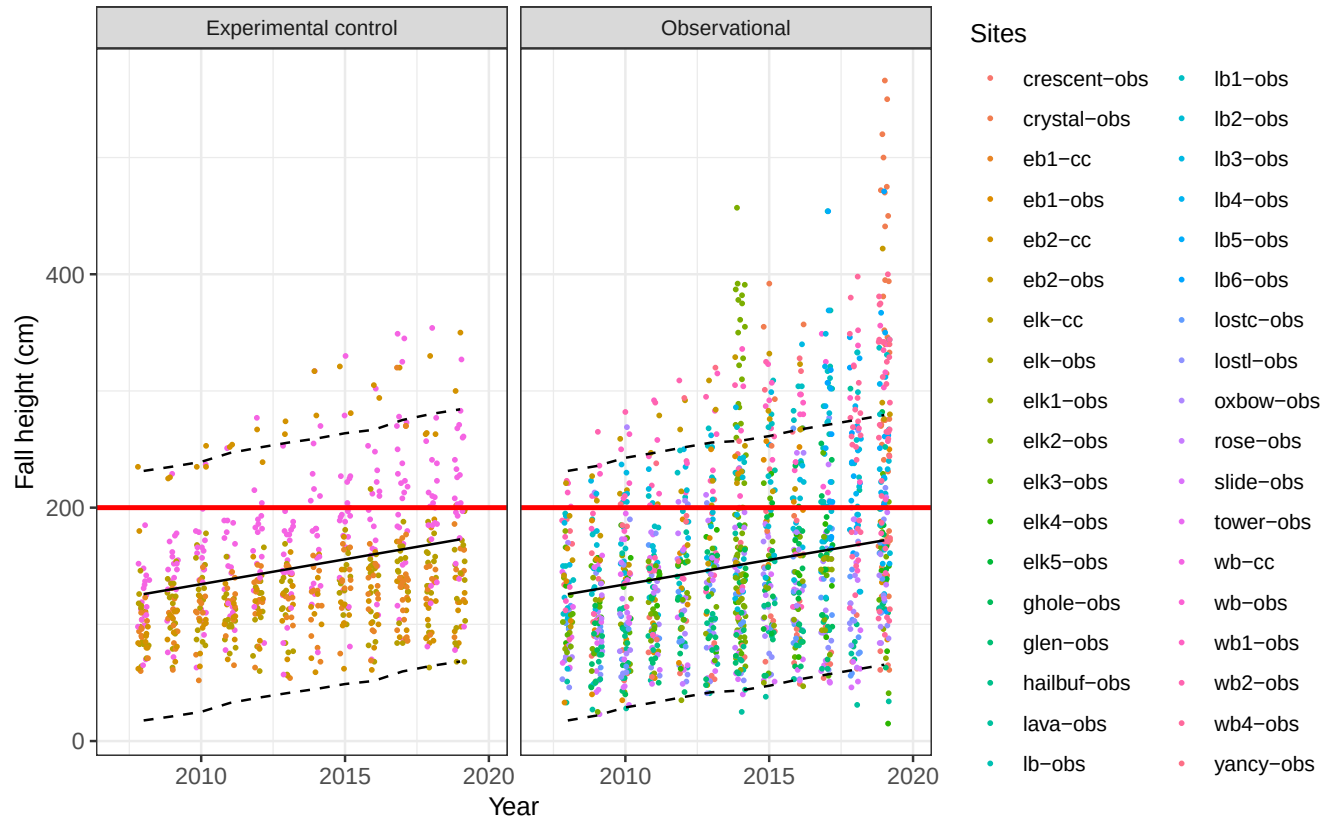


Figure 3: Heights of willows at the end of the growing season on observational and experimental sites during 2008 - 2019. The solid line give posterior predictions of a new mean height and the dashed lines give 95% highest posterior density intervals on the prediction.

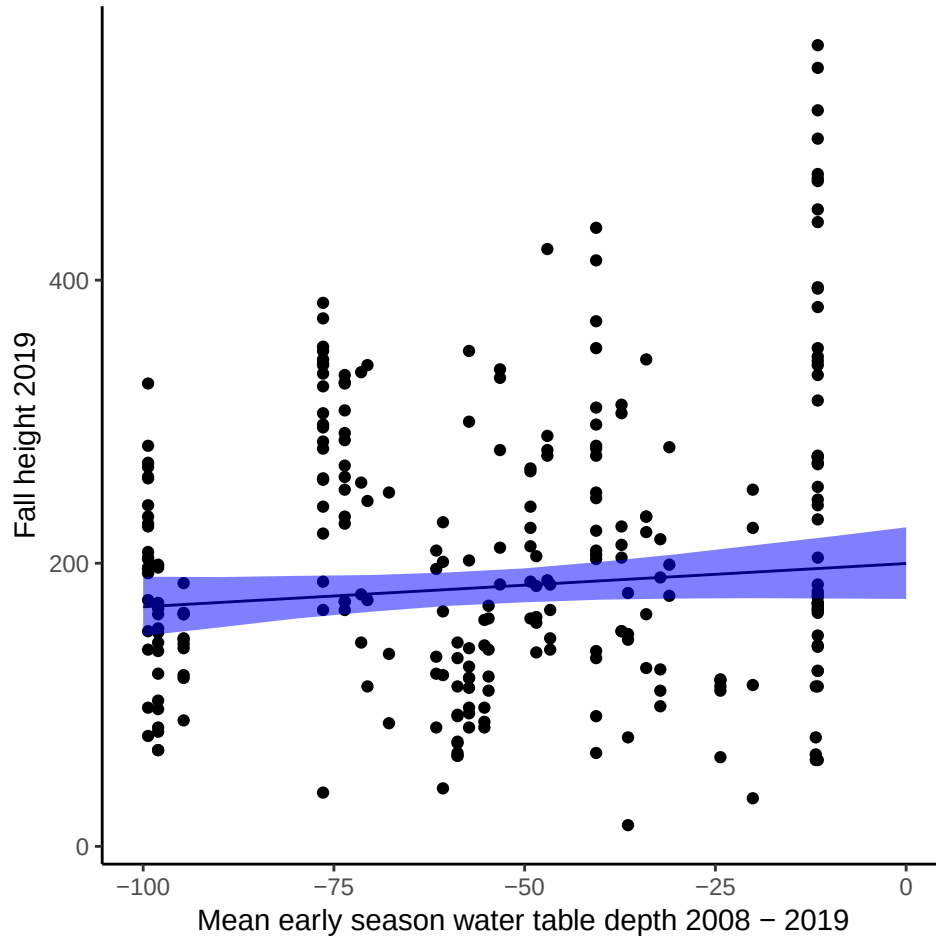


Figure 4: Fall heights of willows during 2019 increased with increasing mean water table depth during 2008 - 2019. Solid line is the prediction of a mean height at a new site. The shaded area shows the 95% highest posterior density intervals.

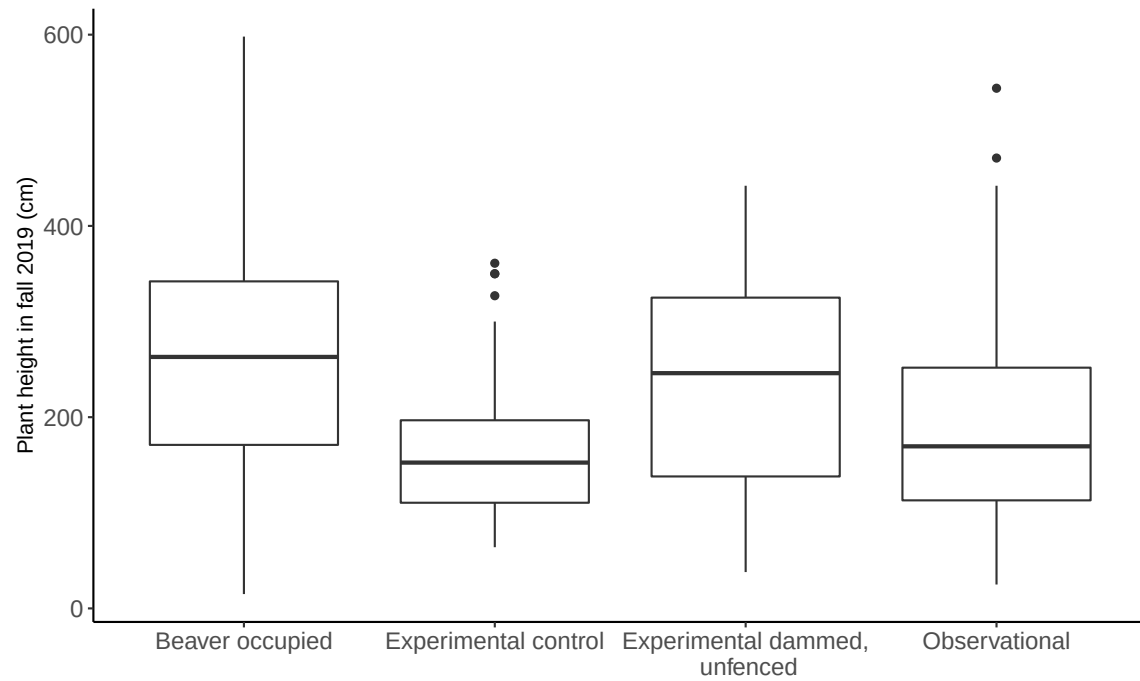


Figure 5: Box plots of willow heights at the end of the growing season in 2019. Sites include areas naturally occupied by beaver, experimental controls (undammed, unfenced), experimental dammed and unfenced plots, and observational plots. All plots experienced ambient levels of browsing. Solid horizontal lines within boxes are medians, boxes show the .25 and .75 quantiles, and vertical dashed lines indicate the tails of the distribution, computed as the most extreme data point which is no more than the range times the interquartile range from the box.

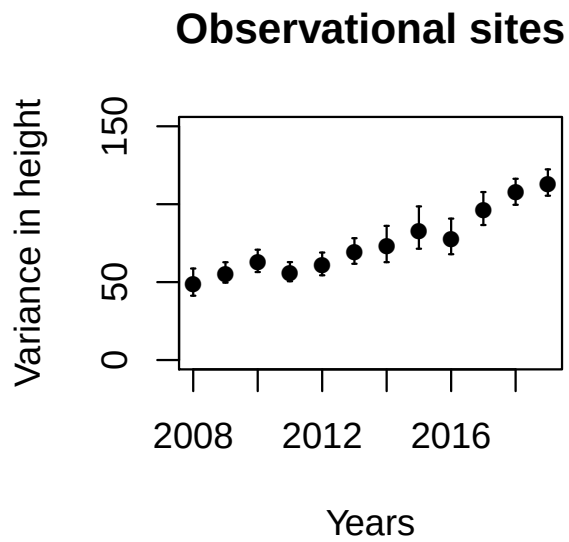
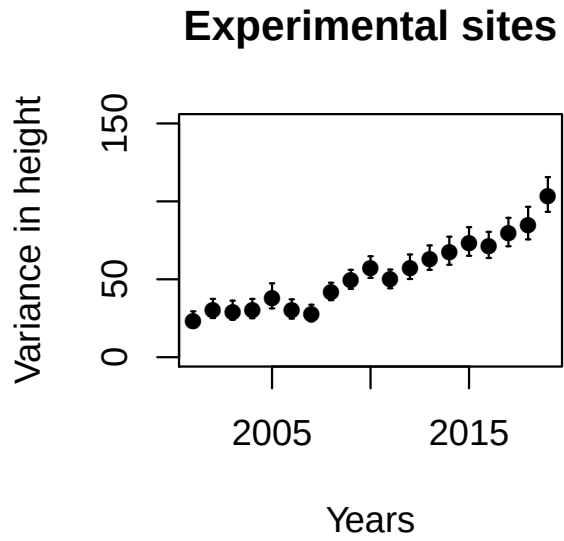


Figure 6: Variance in willow heights on observational and experimental plots almost doubled during 2001 - 2019.

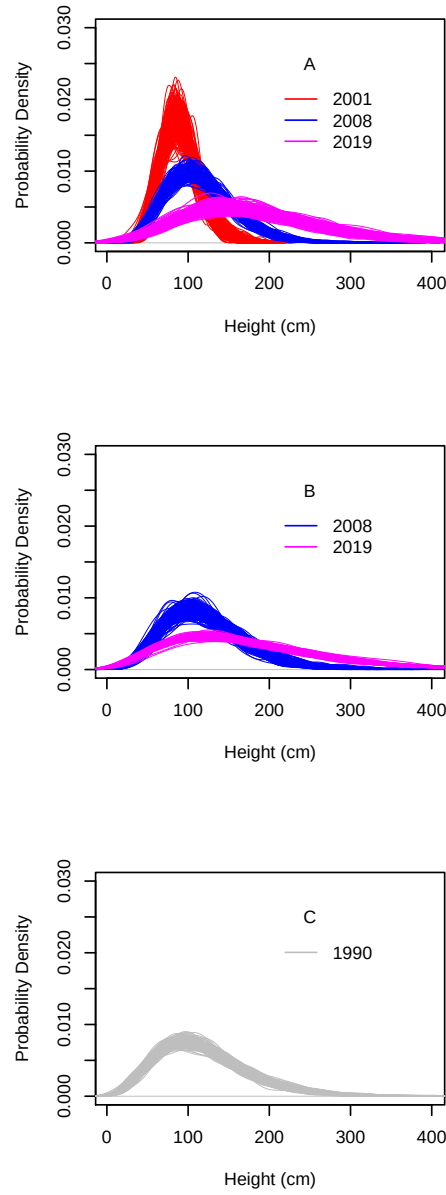


Figure 7: Statistical distributions of heights of and willow from experimental controls (**A**), from observational sites (**B**), and from from pre-wolf data of Singer et al. (1994) (**C**).