

How does experimental warming effect the rate of herbivory and fungi on host grasses?

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Abstract

As there is an increase in greenhouse gasses, it is causing direct and indirect changes in plant interactions with herbivory and fungal pathogens. By imitating the increase in climate change through temperature, it is possible to observe the indirect effect it has on herbivory/fungal pathogens on their plant hosts. In collecting data on three grass species, it cannot be concluded that there is an effect of warming on herbivory/pathogen damage on plant hosts however there is a pattern seen in all species the different plots. The accumulation of herbivory/pathogens in warmed and controlled plots have significance at various time interval of different species. Weather could be affecting the herbivory and pathogens at different plots which can be a reason for the pattern across plots.

Introduction

In the last 100 years there was a 0.6°C increase in global average temperature and it is expected to increase (Root et al. 2003). Such global climate change may have already directly and indirectly affected biota. For instance, changes in precipitation, temperature, soil nutrients, and CO_2 can all impact the primary and secondary chemistry of plant tissues and drive phenological changes (Tylianakis et al. 2008, Ayres et al. 2000). This change in plants, directly caused by climate change, can indirectly affect the plants' interaction with herbivores and pathogens. Rust fungi require moist surfaces and increased humidity (Dukes et al. 2009) however plant pathogens are directly affected by climate change via increased growth and reproduction, changes in infection phenology, and transmission rates (Gilbert 2002). Similarly, pathogens are indirectly affected by climate change via changes in host nutrient status, susceptibility to pathogen attacks, and phenology (Dukes et al 2009, Gilbert 2002). With climate change, herbivore damage and the number of different species attacking their host has increased (Roy et al. 2004). Moreover, climate change has increased insect consumption and developmental times, and has impacted populations by affecting fecundity, survival, and generation time (Dukes et al. 2009, Roy et al. 2004).

Plant-enemy interactions can be altered when the interacting species have responded differently to warming (Dickson et al 2010). Climate change can interfere with host-predator interactions of pea aphid and its fungal pathogen change where susceptibility decreases with an increased temperature (Traill et al. 2010). Furthermore, models suggest that a warmer climate will decrease the generation time of insects, increase insect survival in the winter, and cause species to shift their range toward higher elevations (Dukes and Mooney 1999). For instance, there was an increase in mountain pine beetles that has reduced its generation time from two years to one year because of warming trends, which in turn has increased fungal transmissions (Logan et al. 2003, Parmesan 2006). Roy et al. (2004) found more herbivores than pathogens were found in plots that melted earlier, this did not translate to a universal response of herbivores and pathogens to the environment, with new species found at different time intervals. In this way, herbivores and pathogens present heterogeneous responses to a changing environment.

The effect herbivores have on plant diversity and communities depend on the abundance and type of herbivore present (Olf and Ritchie 1998). Wilf and Labandeira (1999) found that plants had higher frequencies and more varied types of damage in the paleo-ecological record, which they attributed to warming in the Paleocene-Eocene transition. Maron and Crone (2009) found that herbivores had greater effects on plant growth at lower plant densities and have adverse effects on seed recruitment, which may feedback to influence plant abundance. Moreover, herbivores that prey on tree seedlings may limit the distributions of some tree species to locations with specific soil conditions (Fine et al. 2009). Similarly, foliar pathogens can

reduce seedling growth, root biomass, and new shoot production and can cause the death of dominant species leading to shifts in plant communities (Gilbert 2002).

Past work has found that herbivore and pathogen life history is dependent on factors like humidity (Bale et al. 2002), which highlights the importance of investigating the interaction between temperature and precipitation in determining species responses to climate change. Plant enemies have short life cycles, mobility, sensitivity to temperature, and the reproductive potential to have rapid impacts on the distribution of plant hosts (Ayres and Lombardero 2000). Many studies have shown an increase in intensity of plant-enemy interaction as temperature rises (Ayres and Lombardero 2000, Roy et al. 2004, Bale et al. 2002) and how other factors such as humidity affect these relationships (Dukes et al. 2009). For this study we predict that there will be a rapid increase of pathogen and herbivory on their host and how different weather events affect these relationships. Our goal is to observe how warming conditions affect the interaction of herbivores and fungal pathogens with plants. Specifically, we ask: 1) What is the effect of warming on the amount of herbivore and pathogen damage on plants? 2) Do rates of damage accumulation vary between warmed and control plots? And 3) What effect, if any, does inter-annual weather variability have on herbivory and pathogen damage in warmed and controlled plots?

Methods

For this study, we utilize a continuously running, 27 (est. 1990) year old warming experiment at the Rocky Mountain Biological Laboratory. The experiment has ten 3x10 meter plots with 3 meter gaps in between to prevent the controlled plots from being affected. Warmed/experimental plots have electric heaters hung 2.5 meters above the long axis of the plots. Experimental and control plots alternate within the row. The heaters evenly distribute heat with 22 watts per square meter. In each plot, we surveyed three grass species: *Festuca thurberi*, *Poa pratensis*, *Achnatherum lettermanii*. Six plants of each species were observed in each of the ten plots and three different leaves of each plant were tagged in different colors to observe the change in the amount of damage over time. The percentage overall damage on the whole plant and the proportion damaged leaves out of 10 randomly selected leaves from a plant was additionally monitored. Surveys were conducted every two weeks of the growing season from June to early August from 2015 to 2017. In all, we recorded plot identity, species identity, plant individual identity, leaf tag color, the percent of herbivory (e.g. aphid, mine, chew)/pathogen (black, rust, powdery mildew) damage on each leaf and the overall plant. We also analyzed the type of damage done (by enemy functional group/guild) to the plant for each damage measurement.

We used generalized linear mixed effects models fit with maximum likelihood methods to analyze the data. Specifically we used glmer in the lme4 package (Douglas et al. 2015) which specifies both fixed (e.g. warming treatment, year of sampling, days since baseline sampling), and random effects (e.g. plot, plant individual for leaf level measurements). Models were fit using a Poisson distribution (percent damage treated as a count). We used a model comparison/information theoretic framework to assess the evidence that a given model is the “best” out of a candidate set of models (Anderson 2008). Models were compared using AICc and model weights based on the AICc, which can be interpreted as probabilities that a given model is the best representation of reality out of the candidate models, though they are not strict/formal model probabilities. Once we had the best model, we extracted the estimates and standard error and used ggplot2 in order to analyze the data (Wickham 2009).

Results

Although whole plant herbivory in *A. lettermanii* does differ significantly between treatments ($p=0.430$; Figure 1a), there is a trend towards higher herbivory in warmed versus control plots. In contrast, whole plant pathogen damage for *A. lettermanii* does not have a noticeable or significant difference between treatments with warmed plots having slightly more damage than controlled (Figure 1b; $p=0.606$). *F. thurberi* does not have an apparent difference between treatments however warmed plots have slightly more herbivory ($p=0.546$; Figure 2a) for the entire plant. Pathogen damage in *F. thurberi* was highly variable in both treatments which could indicate other factors affecting pathogens in different plots ($p=0.590$; Figure 2b) for the whole plant. Whole plant herbivory damage in *P. pratensis* has a noticeable difference in warmed plots, which was more variable in values than controlled plots ($p=0.1655$; Figure 3a). Similarly, pathogen damage for the entire plant in *P. pratensis* is higher in warmed plots, and marginally significant ($p=0.0518$; Figure 3a). Though generally insignificant, there is a general trend towards higher damage warming plots for each type of damage and for all species.

When analysing our second question, we found the full model to be the best to analyze the accumulation of pathogens through time for *A. lettermanii* (Fig 4b). We measured the change over days, warm and controlled plots, year, days:warm, day:year,:year:warm, days:warm:year (full). The full model was best for *A. lettermanii* herbivory where we tested all the parameters (Figure 4a). Herbivory in *F. thurberi* shows variance when observing the parameters with year having having the greatest difference in the accumulation of damage between warmed and controlled plots (Fig 5a). Pathogens in *F. thurberi* has the full model as the best model where it analyzes all parameters (Fig 5b). Herbivory in *P. pratensis* had the full model as the best model and tested all parameters of the model (Fig 5a). *P. pratensis* fungi utilizes the full model as the best model where we analyzed all parameters to understand the significance between treatments (Fig 5b).

Discussion

As global climate change progresses (Root et al. 2003), it is important to inspect the impact warming has on the herbivory/ pathogen interactions with their plant hosts. Because of this we asked the fundamental question of: What is the effect of warming on the amount of herbivore and pathogen damage on plants? Through inspecting our results we've found no statistically significant difference between controlled and warmed plots. Although the results does not show a significant result, they point towards a pattern where warmed plots experience higher damage across the different plant species (Figure 3a, b). There was also a high amount of variation in damage (Figure 2a) that could may be explained by other influences outside the parameters of this experiment. For instance, there was an early season drought which added stress to the plants and caused many deaths. Because of the drought, many of the tillers had to be re-tagged, which could have contributed to inconsistencies in measuring the same tiller every two weeks. A more significant observation in consequence of the drought at the beginning of this study is the potential of it to influence spore production and travel by herbivores/pathogens, which favor high moisture (Roy et. al 2004, Talley et. al 2002). We suspect that aphids or fungi may have increased in reproduction and survival in controlled plots, because these plots do not have the added drought and heat stress from the lamps. There could be other factors influencing the results that best explains the pattern in the data, however more studies need to be done on the effect warming has on herbivory/pathogens.

Experimental warming has shown multiple implications on climate change including alternating in the relationship between plants and natural enemies. Water resources were limited which give plants poor nutritional value, increase of chemical defense, and lower amount of

insects (Inbar 2001). Regardless, herbivores and pathogens had a preference in warmed plots making warmed plants a better host for natural enemies. Our results imply that with an increase in climate change there is an increase in damage on plant and this can have economic importance to study the effects of abiotic factors on plant interaction with their enemies. The combination of climate change and herbivory/pathogens can reduce plant performance and crop yield, which could jeopardize the sustainability and production of agriculture (Grinnan 2017).

In the ggplot2 analysis, there was variability in the significance of the controlled and warmed plots throughout time. Year is the only parameter that has significance throughout all species for both fungi and herbivory; however, there is no clear pattern similar in all species. An external force can be impacting the warmed and controlled plots for the difference species and causing fluctuations in the accumulation of plant enemies over time. Weather has a wide variety of impacts in herbivory and pathogens. It can cause earlier snowmelt thus impacting plant-enemy interactions and it can change the reproductive success of pathogen and insects through change in moisture or precipitation (Roy et. al 2004, Talley et al 2002). The full impact of climate change on plant-enemy interactions cannot be fully determined and more in-depth studies should be conducted on this topic

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Table 1

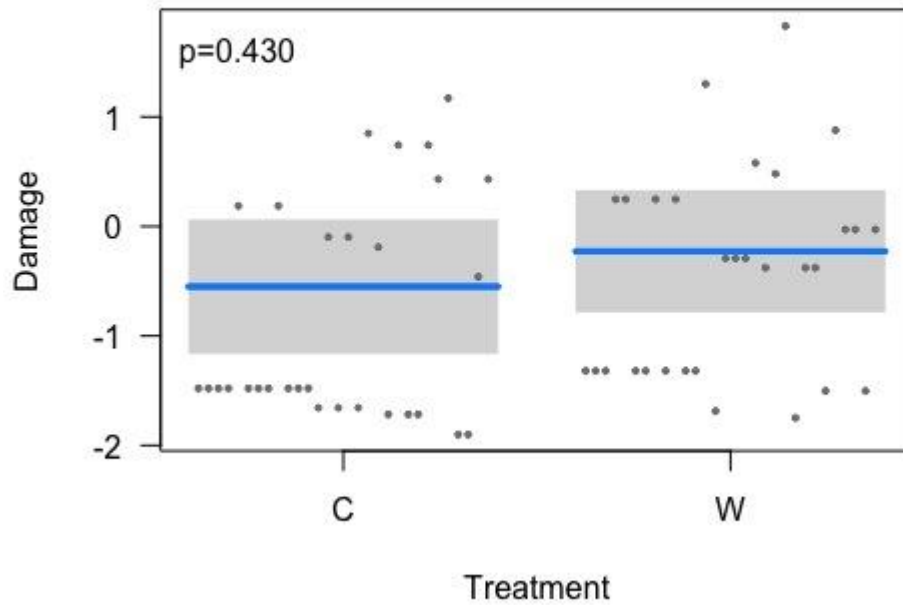
Species	Damage	AICc	Weights	Model	Degree of Freedom	Parameter
<i>P. pratensis</i>	<i>Fungal Pathogens</i>	1903.186	9.994533E-01	<i>Overtime</i>	463	<i>Full</i>
	<i>Herbivory</i>	1190.671	9.977217E-01	<i>Overtime</i>	463	<i>Full</i>
<i>A. lettermanii</i>	<i>Fungal Pathogens</i>	1561.525	9.79714E-01	<i>Overtime</i>	464	<i>Full</i>
	<i>Herbivory</i>	947.4641	8.837771E-01	<i>Overtime</i>	461	<i>Full</i>
<i>F. thurberi</i>	<i>Fungal Pathogens</i>	1373.642	8.283073E-01	<i>Overtime</i>	467	<i>Full</i>
	<i>Herbivory</i>	1426.558	9.050776E-02	<i>Overtime</i>	467	<i>Full</i>

Warming Meadow Site



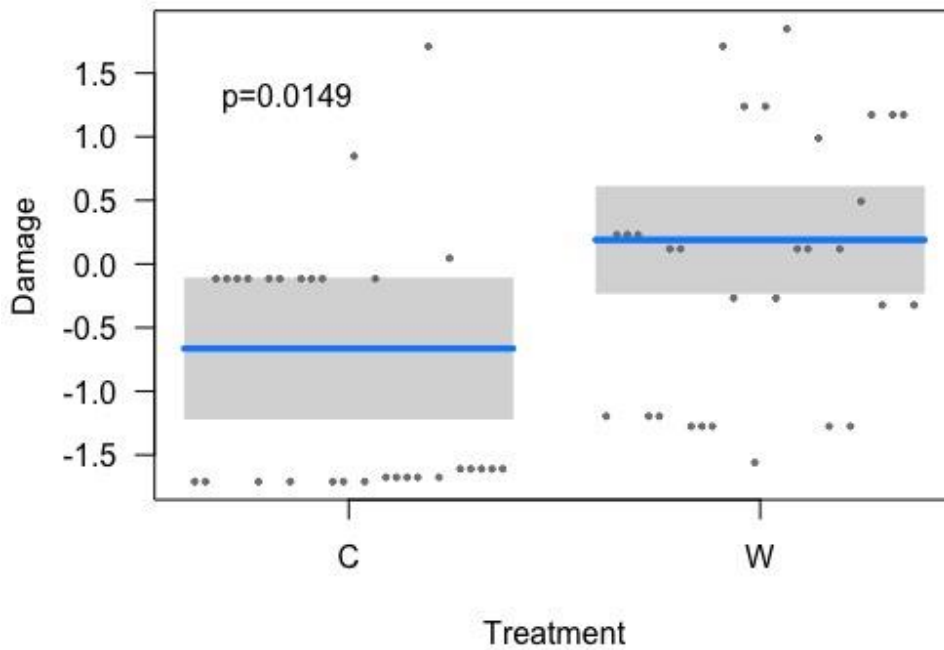
Figure 1

A. lettermanii: Herbivory on Total Individual



A)

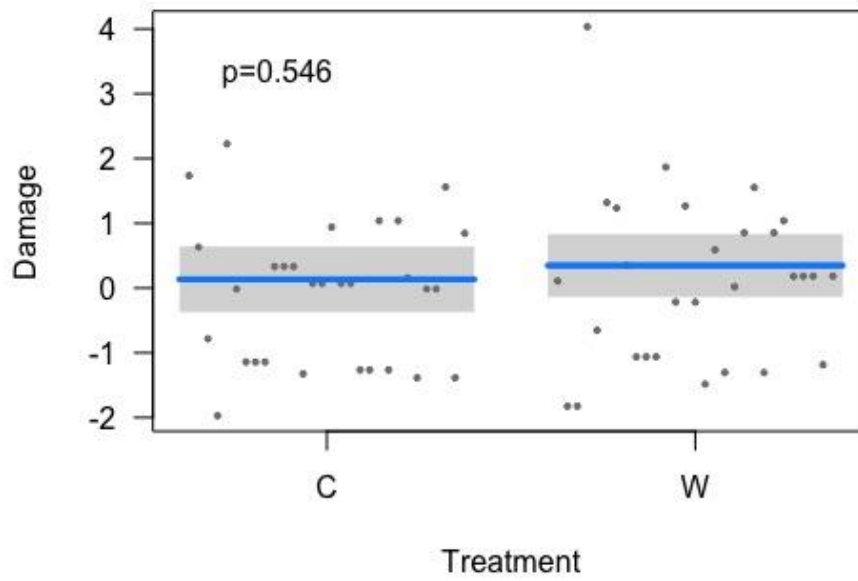
A. lettermanii: Fungal Pathogens on Total Individual



B)

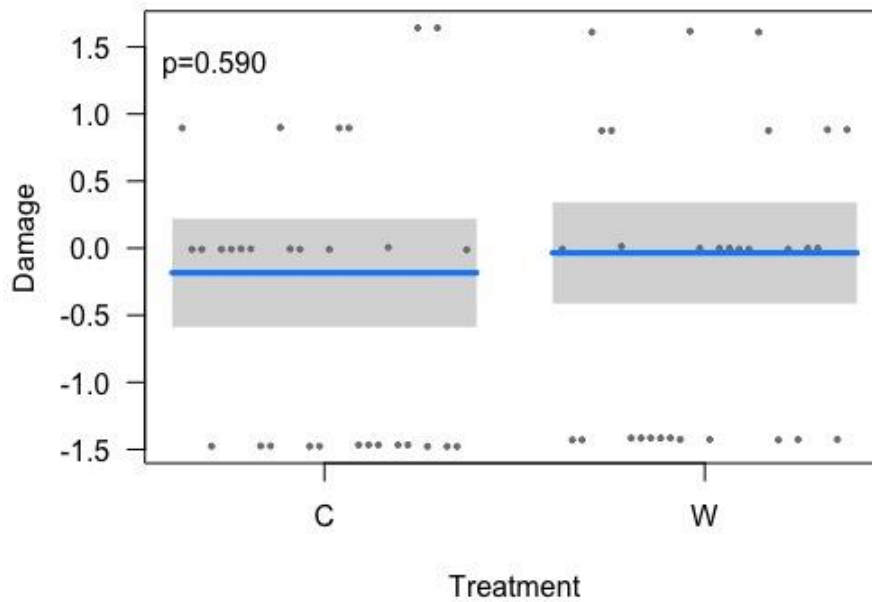
Figure 2

F. thurberi: Herbivory on Total Individual



A)

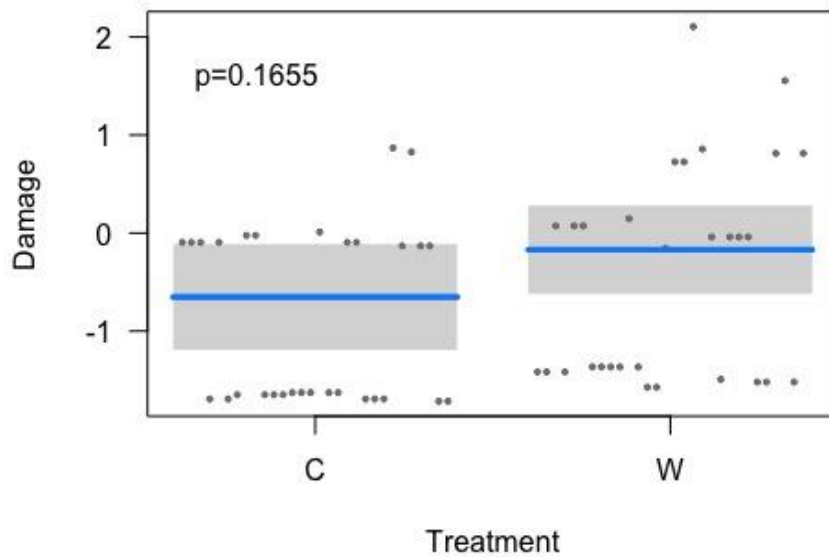
F. thurberi: Fungal Pathogens on Total Individual



B)

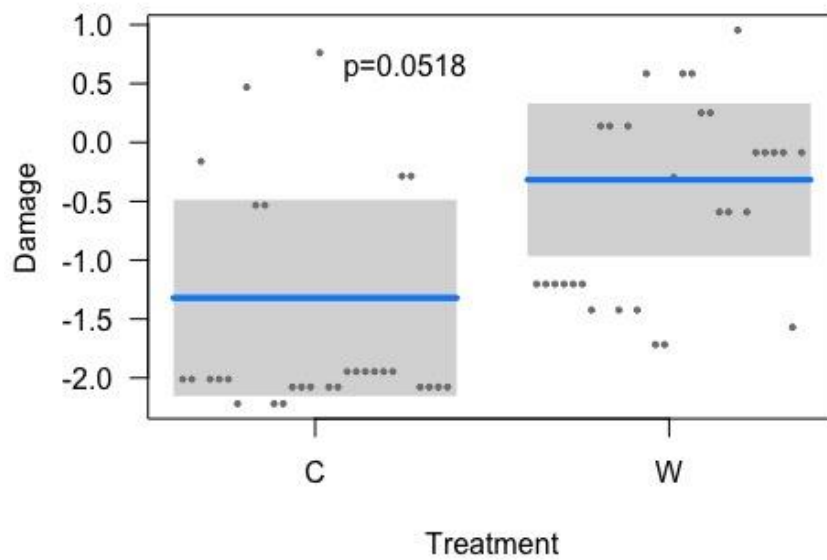
Figure 3

P. pratensis: Herbivory on Total Individual



A)

P. pratensis: Fungal Pathogens on Total Individual



B)

Figure 4

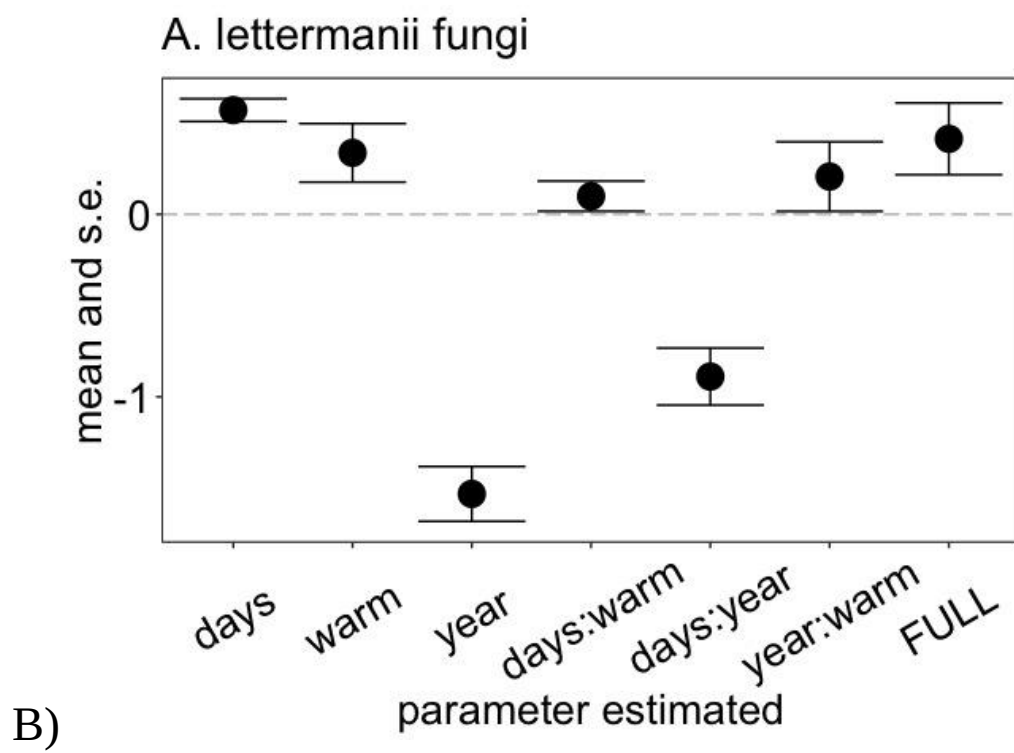
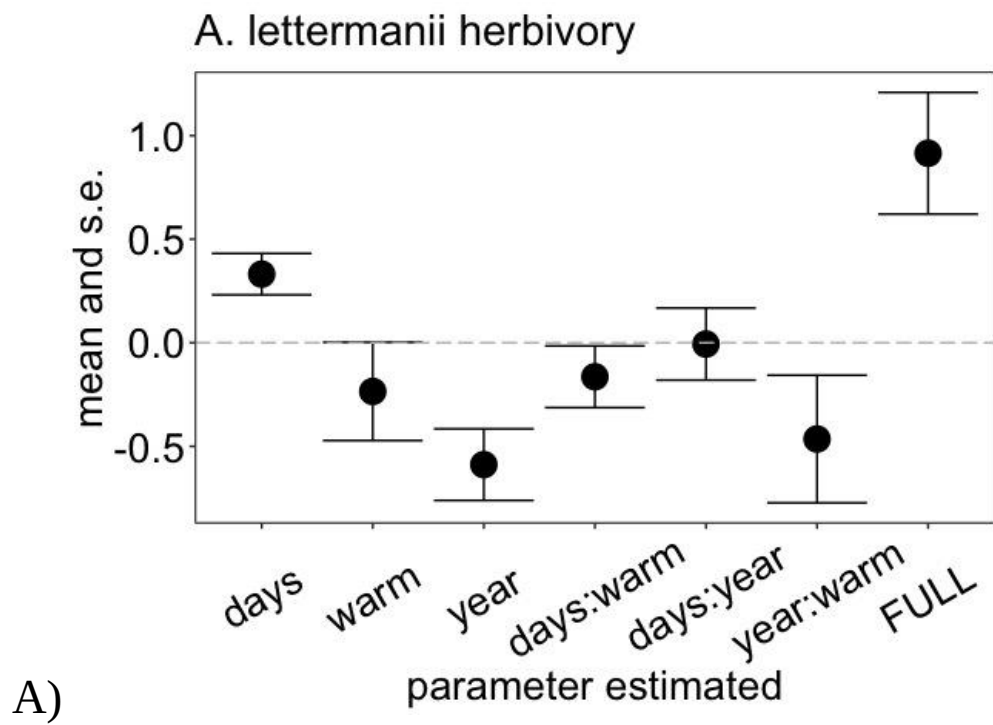


Figure 5

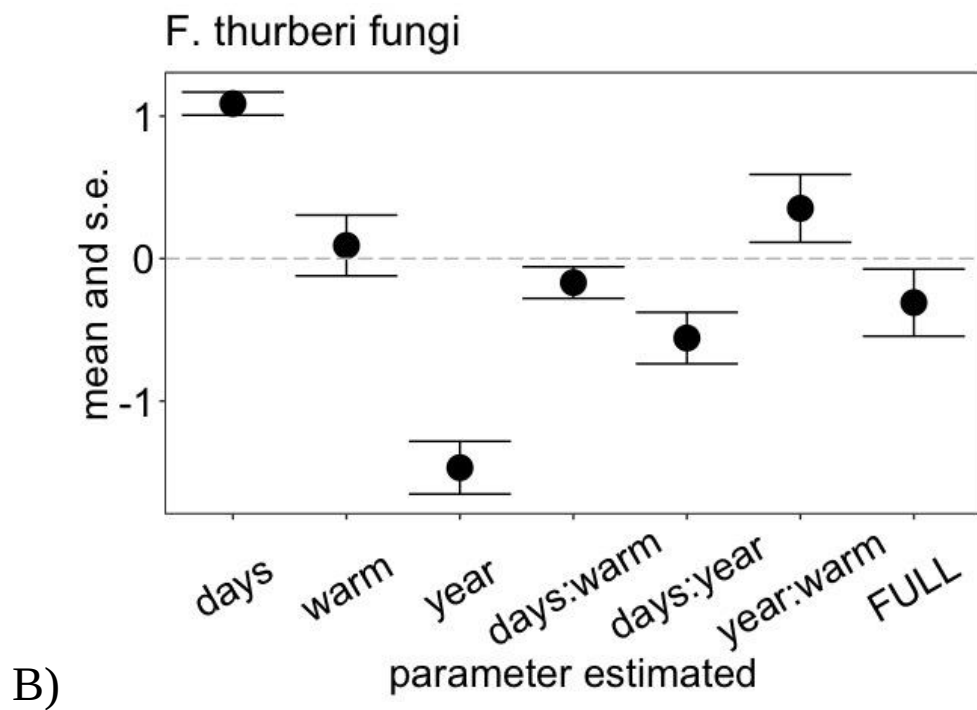
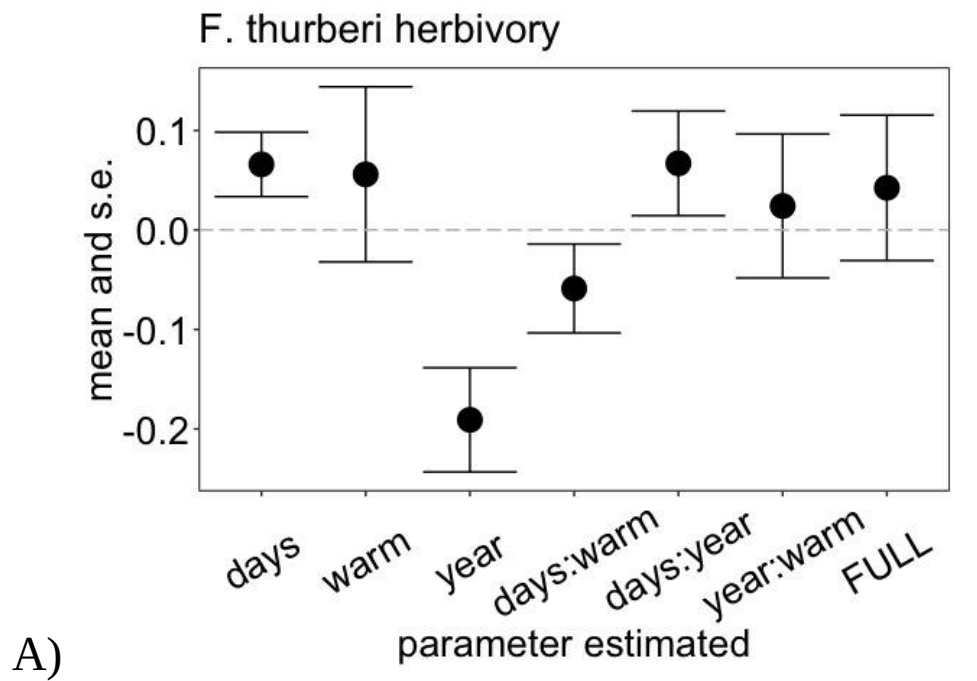
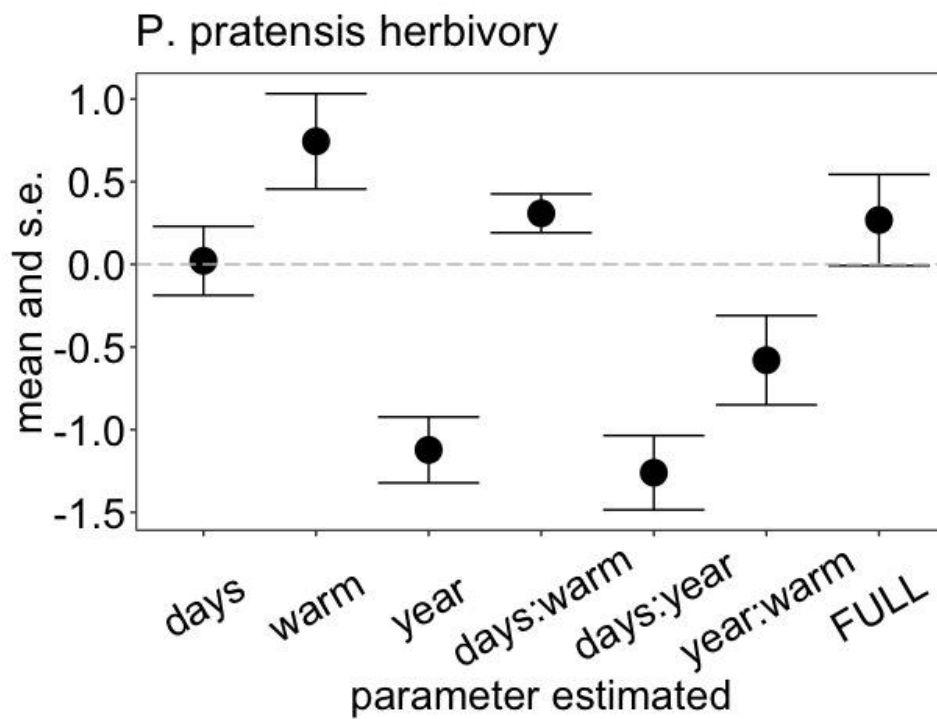
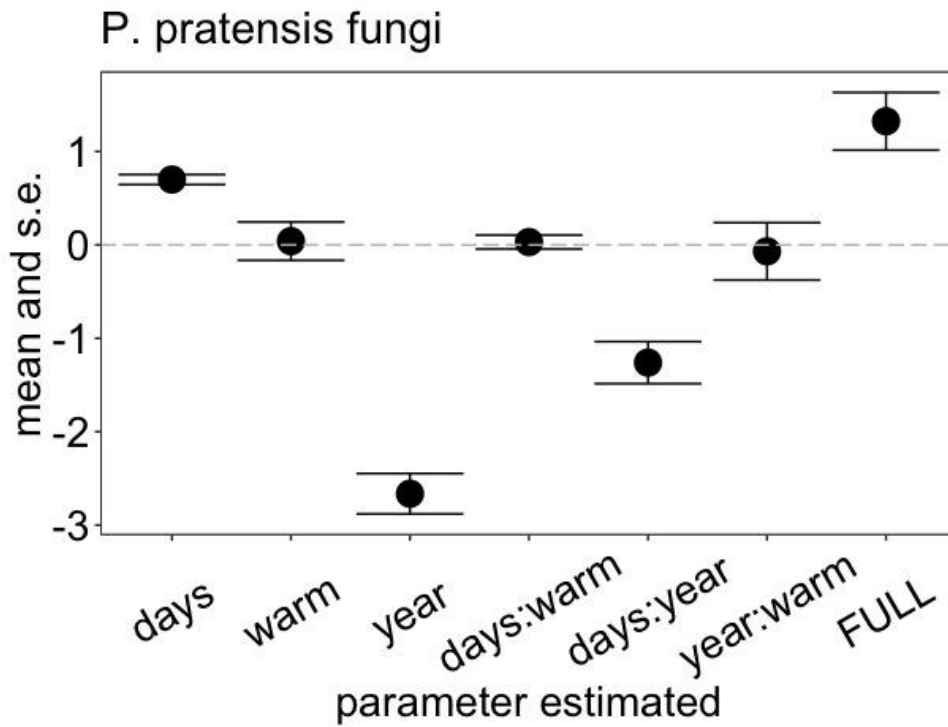


Figure 6



A)



B)

Figure 7

Figures

Table 1 The AICc, Weights, Degree of Freedom (df), parameters, and model of the data collected for all species.

Figure 1 Map of study site

Figure 2 The response of herbivores and pathogens in warmed and controlled plots, as shown the damage between treatments plots for *Acnethrum lettermanii*

Figure 3 The response of herbivores and pathogens in warmed and controlled plots, as shown the percent of damage treatment plots for *Festuca thurberi*

Figure 4 The response of herbivores and pathogens in warmed and controlled plots, as shown the percent of damage between treatment plots for *Poa pratensis*

Figure 5 The change of herbivory/pathogens in different time intervals graphing the mean and standard error (s.e) for each parameter tested in *Acnethrum lettermanii*

Figure 6 The change of herbivory/pathogens in different time intervals graphing the mean and standard error (s.e) for each parameter tested in *Festuca thurberi*

Figure 7 The change of herbivory/pathogens in different time intervals graphing the mean and standard error (s.e) for each parameter tested in *Poa pratensis*