The Effects of Road Fragmentation on Seed Herbivory

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ABSTRACT

Anthropogenic activities such as edging, fragmentation, and road construction and maintenance have a profound effect on ecological systems. Specifically, fragmentation can disrupt food webs by disrupting the feeding behaviours of seed and egg predators and reducing nearby plant diversity. The reduction in plant richness has caused the abundance and diversity of herbivores near roads to also decline. On average, animals that are sensitive to disturbances have a lower herbivory rate near fragmented areas. Therefore, this study will analyze the effects of road fragmentation by measuring the rate of seed herbivory between areas near and far from a road. It is hypothesized that if herbivory is negatively affected by fragmentation, then we will observe more seed herbivory in plots that are further from the road than near the road. A ratio (%) of the number of seeds missing to the original number of seeds placed was measured. The statistical test performed was a 2-way ANOVA test which resulted in a p-value of 0.00001, so the results are significant. Thus, road fragmentation does have an affect on seed herbivory.

INTRODUCTION

Anthropogenic activities include, but are not limited to, land and soil erosion, landscape urbanization and environment deration, deforestation, habitat fragmentation, ocean acidification, industrial pollution, and road construction. Due to the human population rapidly expanding, these activities have increased significantly and have impacted ecological systems (Wilson et al., 2016). Since road construction is the most widespread anthropogenic activity (Bennett, 2017), this study will address the effects of road fragmentation on seed herbivory.

According to Fallahchai et al. (2018), forest fragmentation has shifted animals, mostly herbivores, deeper into forests where they can avoid human interaction. Due to roads cutting through their habitat, it also causes animals to be isolated from plants they rely on for food and shelter. Thus, the adverse effects of fragmentation are more severe for herbivores as they are often specialists, so they have stricter diet and habitat requirements (Rossetti et al., 2017).

Fragmentation can also influence the diversity of plant life near roads. For example, the positioning of canopy trees and the rate of sunlight transference can affect plant species coverage. Therefore, areas where there are very few trees have an open canopy that allows more sunlight to penetrate which leads to the growth of exotic and light-demanding plant species (Anderson et al., 1969). However, an increase in sunlight and new plant species indicate a reduction in soil moisture and space for growth, so native plant species struggle to survive (Delgado et al., 2007). Consequently, herbivores that eat these native plants lose their food source and must move deeper into the forest to find these plant species (Fallahchai et al., 2018).

The harmful effects of fragmentation are not limited to a reduction in species diversity and herbivory rates. Deforestation, fragmentation, and edging may cause natural disasters. For example, in the Amazon Rainforest, deforestation has led to an increase in forest fires during dry seasons. According to Laurence et al. (2007), many significantly bulky regions of the Amazon are negatively impacted by the El Nino-Southern Oscillation (ENSO). The effects of ENSO are a shortage of rainfalls in half-decade intervals causing droughts in these areas which often lead to large forest fires (Philander, 1983). Additionally, since there is less humidity in near-road areas because of the reduced plant life, trees located in these regions are more likely to shed their leaves and/or die from the lack of water. These areas then become dry and susceptible to forest fires (Barlow & Peres, 2008).

Overall, road fragmentation causes a reduction in species diversity and the abundance of herbivores at near-road areas in comparison to areas that are further into the forest. In this experiment, we will measure a ratio (%) of the number of seeds missing to the original number of seeds. Based on literature findings, it is hypothesized that if herbivory is negatively affected by fragmentation, then we will observe more seed herbivory in plots that are further from the road than near the road. Therefore, a positive trend between seed herbivory and the distance from the road can be predicted.

METHODS

This study was conducted in Vancouver, British Columbia, Canada. One group member collected data once a day for seven days between November 13th and November 20th, 2020. Due to COVID-19 restrictions and construction at nearby forests, this group member's home in Vancouver was selected as the study site due to its large front yard and backyard and its location next to a road. This site would mimic a forest located next to a busy road for the study. The front yard vegetation includes one *Prunus pendula* 'Weeping Cherry' tree, two *Hydrangea macrophylla* 'hydrangea', and a few tall *Thuja occidentalis* 'Emerald Green Arborvitae' shrubs on one side. Most of the front yard is the driveway made of concrete. The backyard vegetation includes many tall *Thuja occidentalis* shrubs surrounding the entire yard, a few *Rosa banksiae* 'rose bushes', hydrangeas, grass with clover patches, two *Acer palmatum* 'Sango Kaku' trees, and one *Pseudotsuga menziesii* 'Douglas fir tree'. The backyard has more trees, flower bushes, and grass than the front yard so this mimicked the deeper parts of a forest since vegetation is increasing compared to near the road where vegetation is scarce.

In total, there were 30 sampling sites. Sites #1-10 (experimental group) were placed 2 metres apart in the front yard starting with #1 next to the road and #10 just before the fence connecting to the backyard **(Figure 1)**. These sites were placed on concrete to be 'completely disturbed'. Sites #11-20 (control group A) were placed 2 metres apart in the backyard starting with #11 near the fence connecting to the front yard and #20 at the very back of the yard **(Figure 1)**. These sites were placed on grass to be 'completely undisturbed'. Lastly, sites #21-30 (control group B) were placed 2 metres apart between the front yard and backyard **(Figure 1)**. These sites were randomly distributed on grass or concrete to be ‘slightly disturbed’. For each group, disturbed is referring to the amount of road fragmentation within the study site.

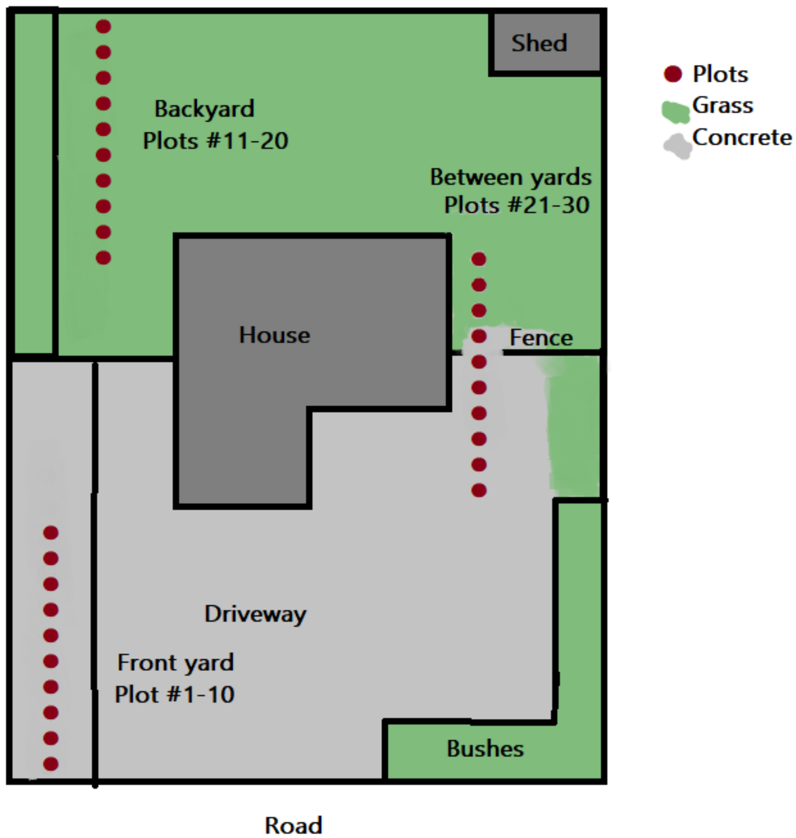

Figure 1. Location of sampling plots. The 30 sampling plots were split into three groups: the front yard (plots #1-10), the backyard (plots #11-20), and in-between (plots #21-30). Therefore, each group contained 10 plots that are each 2 meters apart. The total distance between the edge of the road and the back of the backyard is 60m.

Each plot for the sites was made using grey coloured clay and shaping it into a 4cm diameter circle. The circle was labelled underneath with its site number using a waterproof Sharpie. 10 whole, unsalted sunflower seeds without the shell casing were placed on each clay circle (plot) **(Figure 2)**. Sunflower seeds were used to represent plant seeds in the area as they have been found to be consumed by various local animals. Additionally, the clay was used to help keep the sunflower seeds from blowing away in the wind and show animal traces such as footprints. Since data collection was only performed by one person, it was kept consistent. Data collection occurred at 1:30 PM each day to allow 24 hours for seed herbivory to occur. Upon arrival at each sampling site, the group member first counted the number of seeds present and marked it down in their lab notebook. After counting, if any seeds were missing, she would add enough sunflower seeds to make it 10 again. Additionally, a new clay plot was constructed for each site after each visit.

Figure 2. Image of clay plots #1-10 with ten seeds in each plot.

To compare the levels of seed predation observed between each site, the data was analyzed on an excel spreadsheet. A 2-way ANOVA statistical test was used to compare the means between each site over seven days. This statistical test was appropriate for analyzing the effect of the two independent variables: distance from the road and day.

RESULTS

A 2-Way ANOVA test was performed, and the following pairs of hypotheses were tested as shown in **Table 1**.

Table 1. Hypotheses pertaining to the 2-Way ANOVA test. Hypothesis 1 addresses the effect on the dependent variable, mean relative seed recovery, by the first independent variable, time in days. The second hypothesis addresses the effect of the second independent variable, proximity from road. The third hypothesis addresses whether or not there is an interaction between the independent variables that affect mean seed recovery.

2-Way ANOVA Hypotheses	
Hypothesis 1	H0: μday1 = μday2 = μday3 = μday4 = μday5 = μday6 = μday7
	HA: The means of each day are not equal
Hypothesis 2	H0: μ Front Yard = μ Back Yard = μ Between Front and Back Yard
	HA: The means of each proximity to the road are not equal
Hypothesis 3	H0: There is no interaction effect between day and proximity
	HA: There is an interaction effect between day and proximity

The 2-Way ANOVA resulted in a significant effect of proximity to road of seed herbivory at the $p<0.05$ level for the three conditions [$F(2, 189) = 59.3$, $p<0.00001$], an insignificant effect of time on seed herbivory at the $p<0.05$ level for the 7 conditions [$F(6, 189) = 1.4$, $p=0.21$], and an insignificant interaction between time and proximity to road on seed herbivory [$F(12,189)=0.97$, $p=0.47$]. Based on these results, the null hypothesis 1 **(Table 1)** that the means of each day μday1 = μday2 = μday3 = μday4 = μday5 = μday6 = μday7 are equal cannot be rejected, the null hypothesis 2 is rejected that the means μ Front Yard = μ Back Yard = μ Between Front and Back Yard are equal, and we fail to reject null hypothesis 3 that there is no interaction between variables. Since there were differences found between the proximities using the 2-way ANOVA test, a post hoc test was used to determine where the differences lie.

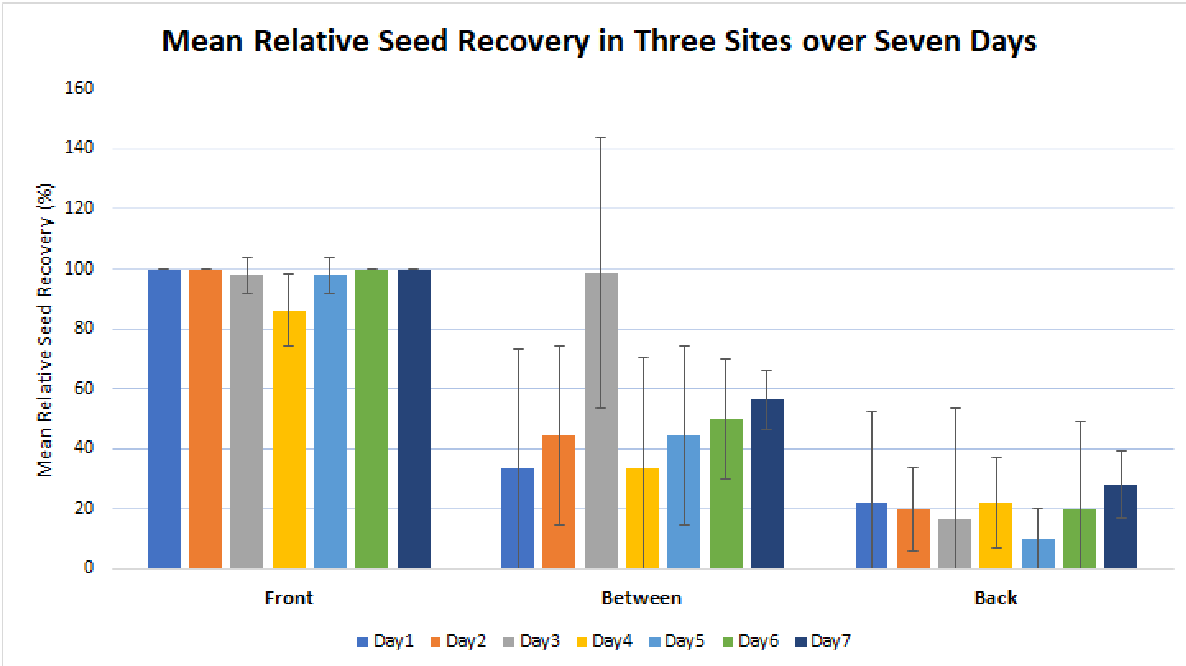

Figure 3. The mean relative seed recovery in three sites over 7 days. The total number of seeds present each day during the observation period on plots #1-30 was normalized over the total number of seeds on the clay plots the night prior to give the mean relative seed recovery. The graph is divided into the three proximities, front yard, between the front yard and backyard, and backyard. The columns represent the seed recovery each day in the three locations and are colour coded for visual ease. The error bars represent the standard deviation of seed recovery (%) on each day and location. The means of the three locations were found to be significantly different based on the 2-Way ANOVA test.

Using excel, post hoc comparisons using the Tukey HSD test indicated that the mean seed herbivory in the front yard (M = 0.23, SD =0.7) was significantly different than the backyard (M = 8.00, SD = 3.8) and between the front and backyard (M = 5.50, SD = 4.8). As well, the backyard and between back and front yard significantly differed **(Figure 3)**.

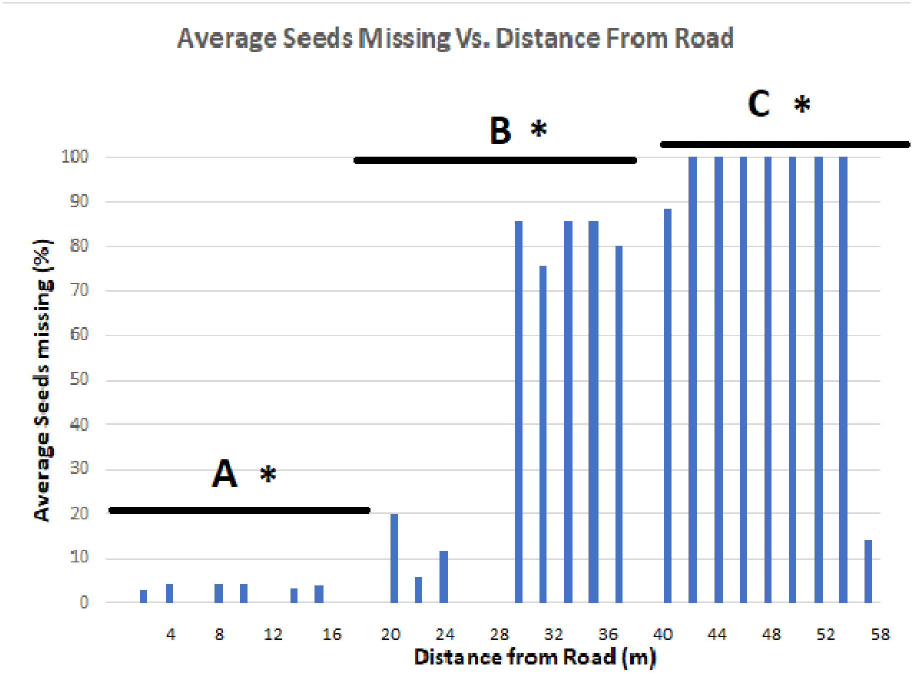

Figure 4. Average seeds missing from clay plots plotted against distance from the road. The column chart was created using the means of data at each plot over 7 days to easily visualize the data. The percentages were found by normalizing the means of seeds missing over the total number of seeds originally on the clay plot. A) shows plots #1-10 in the front yard with a mean of 2.3%. B) shows plots #21-30 in between the front and back yard with a mean of 45.0% and C) shows plots #11-20 in the back yard with a mean of 80.3%. There is a significant increase in the number of seeds missing between site A, B, and C as denoted by the asterisks (*).

A graph was constructed on excel using the primary data from the study of how distance from the road affects herbivory **(Figure 4)**. The sudden increase in seed herbivory could be explained by the gate which acted as a physical barrier between the front and back yard, as seen in **Figure 1**. Based on our post hoc test, we can reject the null hypothesis that the means between sites were equal and are shown with asterisks. Although a comparison was made between each site (front yard, backyard, and in-between) distances also varied within each site (distance between each plot and the road) and are shown for easy visualization. Linear regressions were not able to be made and more data points would be needed to analyze each proximity. Out of a total of 210 clay patties covered with 10 seeds each in our three locations, herbivory was observed to be 2.3% in the front yard and 80.3% in the backyard, as seen in Figure 4. Different types of animal traces were observed over 7 days including footprints and bite marks, indicating a variety of herbivores such as rodents and birds **(Figure 5)**. These traces were seen on plots #10, 16, 20, and 30. However, since the clay was wet for most of the study, many of the animal traces were not observable **(Figure 5)**.

Figure 5. The different types of seed predation that was prevalent during our study. **Left:** represents complete seed predation, as there are no seeds remaining on the plot. **Middle:** demonstrates incomplete seed predation, or the effects of rain and/or wind on the seeds. **Right:** represents the bite marks left on the clay plot by a seed herbivore.

DISCUSSION

Studies conducted by Slabbekroon et al. and Naguib suggest that anthropogenic noise (cars, people, etc.) can negatively affect the communication of animals resulting in their evacuation of the area (Slabbekoorn et al., 2018; Naguib 2013) which would indirectly affect the amount of seed predation in the area. Moreover, as indicated by Fallahchi et al., animals try to avoid contact with humans as much as possible therefore, we observe higher herbivory in areas that are less occupied by humans (backyard) and lower herbivory in areas that are more occupied by humans (sidewalks, roads, and driveways).

After 7 consecutive days of data collection from 30 sampling plots, the results indicate an increase in herbivory rate in areas that are less disturbed by road fragmentation. This is represented by the average number of seeds eaten between the near-road sites being higher than those located further from the road **(Figure 4)**. These results support the hypothesis that higher seed herbivory is observed in plots that mimic the natural environment and are less disturbed, while lower herbivory is observed in plots closer to the roads and are more disturbed.

Ultimately, the results of this study support the initial hypothesis and prediction. However, due to many experimental limitations, the degree of uncertainty is unknown. The main limitation in this experiment is the location. Due to the global pandemic, we were unable to do the experiment in a forest such as Pacific Spirit Park as was initially planned and so, it was assumed that the backyard of a home could resemble similar conditions to a forest. According to the literature, there is a decline in abundance of herbivores near boundaries where fragmentation occurred. Although, this is referring to large forests with thin roads intersecting whereas, at the study site, forested areas are the minority in comparison to the houses and roads. Therefore, re-performing this experiment in a large forest would obtain more accurate results that align with the literature.

Another limitation of the location is the size of the area. This study was conducted at three proximities within a relatively small area. Additionally, since each plot is only 2 metres apart, it can not be stated for certain that an herbivore finding one plot would not lead to it finding other plots at that transect or other transects. Thus, increasing the distance between the transects and each of the plots would be needed to test the correlation between distance and change in herbivory. Alternatively, repeating the experiment with more than three proximities and a greater number of plots may provide better evidence to support the hypothesis.

Plots were made of clay to attempt to see what herbivore species were present based on the prints and/or marks they left on the clay. However, in most cases we were unable to fully capture these traces and failed to precisely identify the herbivores consuming the seeds. Regardless, in some cases, it was successful since some clay pads were being torn apart by what presumably could be rats, squirrels, and/or raccoons. For future studies, although expensive, it would be useful to install cameras that can capture the nature of these consumers to understand the local diversity of the herbivores.

During the days of data collection, there was heavy rainfall and wind that caused the clay pads to be flipped over and/or seeds to potentially be washed away. This could have affected typical herbivore behaviour, or the number of seeds recorded to be eaten at that location. For future experiments, forming the plots out of material that is resistant to being moved by the wind and/or stops seeds from being washed away will ensure a more precise result. Moreover, the results from the 2-way ANOVA showed that there was not a significant difference between seed herbivory over the 7 days. Data collection occurred in late fall/early winter and was only for 7 days. Since different seasons have a variety of effects on the behaviour of animals, it may have influenced herbivory. 7 days may also not have been enough time to capture accurate data. Therefore, repeating this experiment in different seasons and during a longer time-period would confirm if these factors influenced results.

The type of seeds present in an area may also affect herbivory rate. Sunflower seeds were chosen in this experiment since most herbivore species prefer these oil-based seeds (Pilson, 2000). However, sunflower plants are not where the experiment was conducted, and this may have affected the results since there could have been herbivores that do not consume sunflower seeds. This could have resulted in a lower herbivory rate than there should have been based on the number of herbivores in the area. Future studies should use seed producing plants native to the study site and test the effects of herbivory using seeds produced by those plants. This will increase the accuracy of the observed results and demonstrate the effects of herbivory for the native plants of that region. Alternatively, since specialist herbivores are most vulnerable to fragmentation, future experiments should use a variety of seeds to account for herbivores that eat those specific seeds rather than be exclusive to herbivores that eat sunflower seeds. This could obtain results with greater accuracy on herbivory rate (Rossetti et al., 2017).

CONCLUSION

The findings demonstrate that there is a significant difference between the rates of seed herbivory between the front yard (near-road) and the backyard (further away from the road). On average, a higher ratio (%) of the number of seeds missing to the original number of seeds placed was recorded for the plots that were positioned in the backyard. This indicates that there is greater seed herbivory observed in plots that are further away from the road than those near the road which supports the hypothesis. Based on the results, it can be concluded that road fragmentation negatively impacts the rate of seed herbivory observed in an ecological system.

ACKNOWLEDGEMENTS

We would like to thank Dr. Celeste Leander, as well as Anne Kim and Tessa Blanchard, for their exceptional support and guidance during the course of this research. We would also like to extend our gratitude to Biology 342 which enabled us to conduct this research.

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