Preregistration

Arthropod Diversity in Regrown Urban Grass Environments

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Study Information

Title	Arthropod Diversity in Regrown Urban Grass Environments
Description	The key objective for this study is to create a comprehensive community structure of arthropods throughout grassland regrowth. Obtaining a weekly breakdown of arthropod community structure will allow me to analyse the data to answer my hypotheses and also look for trends or outliers in the data that may allude to an unexpected outcome of grassland regrowth.
Hypotheses	Additional Niche Hypothesis (directional): As grasslands regrow, the height of vegetation will increase the spatial niche available for arthropods to habituate. Literature outlines the positive relationship between arthropod abundance and an increase in canopy cover (Blaise et al., 2022), therefore; I hypothesize if urban grasslands

experience discontinued mowing, arthropod diversity will be altered as available niche zones increase.

Plot Size Hypothesis (directional): Grassland plants will diversify over time as new plant species colonize the area and pre-existing plants are able to thrive without disturbance. The size of the regrown plot may have an influence on plant diversity because dispersing seeds are more likely to land in a larger area and spreading plants can efficiently colonize the untouched grass area. Additionally, large areas of grass are more likely to contain pre-existing plant diversity compared to a small patch of grass. Moreover, larger grass plots are more likely to collect dispersing plant seeds and contain pre-established plant diversity, which will increase plant diversity fastest during grassland regrowth. Since an arthropod population can only be as diverse as the plant life available to support them (Haddad et al., 2001; Joern & Laws, 2013), I hypothesize if urban grassland plots experience discontinued mowing, arthropod diversity will scale with plot size.

Proportional Diversity Hypothesis (directional): As grasslands regrow, the plant community arthropods rely on will diversify and cause changes to arthropod community composition. Literature outlines how consumer diversity relies on the diversity of resources available to them, therefore; changes to the plant community will reflect in changes to the supported arthropod community. Generalist and specialist arthropod populations will change as their grassland environment diversifies and can support additional specialist arthropods. I hypothesize if urban grasslands experience discontinued mowing, generalist and specialist arthropods will experience changes in diversity throughout grassland regrowth.

Predicitons

Additional Niche Prediction: Untouched grasslands will become more diverse over time, which increases the livable area for arthropods as new plant species grow tall and colonize the regrown grassland. Additional vertical niche zones increase the abundance of ground- and canopy-dwelling arthropods (Blaise et al., 2022), which increases arthropod community stability because generalist populations, and their essential ecosystem functions, are maintained by species richness (Hurd, 2008). Increased stability of the grassland ecosystem will promote arthropod diversity because the carrying capacity will increase and the stable ecosystem will support new arthropod species. I predict arthropod diversity will increase throughout grassland regrowth as plant species diversify and are better able to support arthropod diversity with additional niche zones.

Plot Size Prediction: Under normal seed dispersal conditions, dispersing seeds are more likely to land in a large plot, compared to a smaller plot. Additionally, large plots are more likely to contain pre-existing plant diversity. Simply based on the probability of large plots collecting more dispersing seeds and containing greater plant diversity than smaller plots, I predict that larger plot sizes will have the fastest growing arthropod diversity. Since arthropod diversity is directly correlated to the plant life available to them (Haddad et al., 2001; Joern & Laws, 2013), the plots with the most plant diversity will have the most arthropod diversity. The largest plot size of 17x17m will have the fastest growing arthropod diversity in initial grassland regrowth because the larger area is more likely to collect dispersing seeds and regrow a variety of plant species. The smallest plot of 3x3m will have the slowest growing biodiversity as it will take longer for arthropod and plant species to discover and colonize the regrown patch. The intermediate patch size of 10x10m will scale more closely to a reciprocal correlation between grassland regrowth and arthropod biodiversity.

Proportional Diversity Prediction: Generalist arthropods maintain ecosystem functions and can thrive in a wide range of conditions (Hurd, 2008). As grasslands regrow, their population will increase in richness as the environment's carrying capacity increases. Moreover, I predict the generalist arthropod population will steadily diversify throughout grassland regrowth as plant species diversify and can attract additional types of generalist arthropods. Alternatively, specialist arthropods often rely on a single plant species and are sensitive to changes in the environment, making specialist arthropod populations hard to maintain (Hurd, 2008). I predict specialist arthropod diversity will remain consistent throughout grassland regrowth until a threshold of plant diversity is reached. Once plant diversity begins to increase, specialist arthropod diversity will reciprocally increase because the new, specialist plants will attract specialist arthropods to the grassland. Additionally, as the grassland regrows, generalist populations will increase in species richness and create a stable ecosystem conducive to supporting specialist arthropod populations. Finally, in an urban grassland environment, the community composition of arthropods will have more generalists than specialists, even when plant species diversify. Many generalist arthropod species serve the same ecosystem function and can replace one another seamlessly. Generalists are responsible for maintaining a stable ecosystem by having many species completing the same tasks, so if there is a disturbance, the ecosystem maintains function. Therefore, generalists will have the greatest diversity throughout grassland regrowth as they can survive in a wide range of environments and are essential for ecosystem functioning.

Design Plan

Study type

Observational Study. Data is collected from study subjects that are not randomly assigned to a treatment. This includes surveys, natural experiments, and regression discontinuity designs.

Blinding

No blinding is involved in this study.

Study design

This study is conducted in an urban grassland located in Cambridge ON, at the Cambridge Southwood Secondary School. There are 3 grass plots in sizes of 9m2, 100m2, and 289m2 that will be regrown in the school yard at a distance of 18m apart from each other. The grass plots will remain stagnant without mowing for the duration of this 2-year study. Weekly samples are taken from May-October because arthropod communities are not active in the winter. Additionally, only the surrounding areas of the plots will be mowed as per usual. Malaise traps placed in the center of each plot will trap arthropods to be preserved for metabarcoding. No human management will be used to support the regrowth of the grass plots as this study focuses on the unbiased arthropod recolonization process.

Randomization

This study does not involve randomization in the study design. Randomization will be used throughout lab processing of samples in the stages of PCR and normalization.

Sampling Plan

Existing data

Registration prior to any human observation of the data. As of the date of submission, the data exist but have not yet been quantified, constructed, observed, or reported by anyone - including individuals that are not associated with the proposed study. Examples include museum specimens that have not been measured and data that have been collected by non-human collectors and are inaccessible.

Explanation of existing data

The data used in my research was collected before and during my master's project thus far. It has not been viewed since my advisor recieved it as we are waiting to have all the data before viewing and completing analysis with it.

Data collection procedures

Malaise traps are widely used in the scientific community as they construct an accurate representation of the local arthropod biodiversity in an environment and are specifically effective at targeting flying arthropods (Skvarla et al., 2021; Trap Responses of Flying Insects - 1st Edition, n.d.). A malaise trap is placed in the center of 3 isolated grass plots, ranging in size from 9m2-289m2, where it collects arthropods visiting or passing through the regrown grass plots. Each malaise trap is identical and is secured to the ground using thin rope and stakes that are maintained weekly. The malaise trap is designed with mesh netting to encourage the already innate tendency of insects to climb when met with a barrier (Skvarla et al., 2021). The arthropods that climb to the top of the trap are preserved in a bottle of ethanol throughout each 1-week sampling period. Ethanol is used to preserve arthropods as it eliminates the enzymes responsible for enzymatic breakdown of DNA, while also drying out tissues to reduce damage to DNA (Marquina et al., 2021). Ethanol bottles are exchanged weekly with a new bottle filled with about 450ml of ethanol, then stored at room temperature in the Steinke lab to be processed for metabarcoding. The arthropods present in each weekly sample will construct a community composition that will be analysed throughout this study.

Sample size

There are 3 plots of varying sizes being analyzed in the study. The sample size is 3.

Sample size rationale The sample size of this project was chosen by my advisor before I was in contact with him to complete my thesis project. There are also space and monetary constraints for this project, which is why 3 plots is the chosen sample size.

Stopping rule I am using an exact sample size of 3, therefore no stopping rule is needed.

Variables

Manipulated variables In my research, I am identifying arthropod species using metabarcoding. The results we obtain from metabarcoding state a 0 or 1, depending on if the species' DNA is present in the sample (1), or not present in the sample (0).

Measured variables The measured variable will be a 0 or 1, depending if an arthropod species is present in the sample (1), or not present (0).

Indices I not not have any planned indices.

Analysis Plan

Statistical models I will use a one-way ANOVA test to determine if plot size has a statistically significant impact on arthropod diversity. I will compare the means of each plot size's arthropod diversity using the ANOVA test. I will also be using several regression analyses to determine if the relationship between plot size and arthropod diversity is significant.

Transformations At the moment, I no not believe this study requires any additional transformations.

Inference criteria As per most studies, the standard $p<0.05$ represents statistically significant results, which is what this study will base its analysis results from. This standard value will be used in all analyses.

Data exclusion	Outliers will be mentioned in analyses, but excluded from formal analyses as they will skew results.
Missing data	Missing data will be mentioned in analyses and explained if it causes a large skew in results. Otherwise, in my study, a missing week of data likely will not heavily impact the analyses.
Exploratory analyses (optional)	This study is partly exploratory as we are looking for changes in diversity of arthropods as the plot of grass regrows. We are looking for any interesting changes and if a change in diversity occurs that we had not predicted, it will be explained as an exploratory finding.

Other

Other (Optional)	

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