Examining soil fertility effects on gall abundance and diversity in a tallgrass prairie

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Abstract

Plant galls are abnormal modifications of plant tissue by an external organism to create a living chamber and feeding area. Plant galls are made by mites, viruses, fungi, bacteria, and insects and they come in a variety of sizes, differentiation, and location on a plant. Soil, wet to dry gradients, and vegetation type have all been proposed as explanations of this gall abundance and diversity. Soil seems to have been somewhat underemphasized in importance by many previous studies even though soil controls nutrient and water availability. In this study, higher soil fertility was hypothesized to result in lower galling due to plants having better defense and being less stressed. An observational approach was used to explore this hypothesis by using both gall abundance and diversity to test it. Several plots were searched for galls and soil samples collected from these plots. Overall, upland and lowland sites had similar soil nutrients and gall abundance and diversity. Interestingly, gall abundance and diversity was surprisingly high with over 500 galls found and nearly 20 morphotypes encountered. The results did not support the idea that less fertile plots will have more gall abundance or diversity. An alternate explanation supported by the data is that plant composition controlled galling abundance and types very strongly as goldenrod and burr oaks dominated the diversity of galls and their abundance.

Introduction

Insects and plants interact in many direct and indirect ways. These interactions include herbivory, pollination, decomposition, and range from mutualistic to antagonistic relationships (Taiz et al, 2018). One strange and often unnoticed parasitic interaction are plant galls. Galls are intriguing and often cryptic, but once noticed they become apparent in plant communities in any habitat around the world. Galls are on every continent except Antarctica and several thousand species exist in North America alone (Felt, 1940; Larew, 1981). Additionally, galls can be agriculturally important, such as the hessian fly which creates stem galls on wheat plants that cause significant economic damage (Flanders et al, 2014).

Galls are abnormal internal or external growths of a plant that are caused by another organism. Organisms that cause galls include mites, bacteria, fungi, viruses, nematodes, and insects. The organism initiates the gall formation by releasing hormones or chemicals that alter plant hormone levels such as cytokinin and auxin (Mapes & Davies, 1998; Larew, 1981). The galling organism either lives completely inside the plant or creates a pouch or like structure on the outside of the plant. Galls are categorized based on location, degree of modification, and by what organism causes it (Larew, 1981). Common gall locations include seeds, flowers, roots, leaves, and stems. Two notable examples are maple bladder galls which are reddish lumpy bumps on upper surface of maple leaves and grape phylloxera galls which are green lumps on grape leaves. Both gall types can be numerous on one leaf or several (Eiseman & Charney 2010). Historically, gall makers have existed at least 300 million years as evidenced by abnormal extensions of plant fossils such as those found in the Illinois Basin (Grimaldi & Engel, 2005).

Galls reduce plant fitness by causing reproductive losses or stunted growth (Wise &

Abrahamson, 2017). However, plants have ways to deal with galls, including tolerance or resistance (Wise & Abrahamson, 2017). Tolerance means allowing the gall or galls to form. Resistance means hindering or killing the galling organism. Both strategies are found throughout the plant kingdom and sometimes the same plant species uses both. For example, *Solidago altissima* has some genotypes that are very resistant to goldenrod ball gall flies while other genotypes are highly galled. This plant sometimes will use necrosis to kill the fly larva and some individuals have a bent apical stem in the early summer that hinders oviposition by the ball gall fly and rosette gall midge (Wise & Abrahamson, 2008). Overall, the effectiveness of plant defenses against galls depends on genotype, plant species, abiotic stresses like nutrients and drought, age, and the local plant community (Wise & Abrahamson, 2017; Wool, 2004).

Several hypotheses have been proposed to explain why galls are more abundant or diverse in certain habitats. Most hypotheses predict that xeric habitats will have more gall diversity such as an Argentina transect study (Fernandez et al, 2002; Blanche & Ludwig, 1998). Other hypotheses and studies found soil fertility as a better explanatory factor and others observed woody versus herbaceous diversity to be the driving factor such as in Big Bend National Park (Blanche & Ludwig, 1998). In addition, galls are strongly controlled by local factors, landscape features, dispersal mechanisms, local predator and parasite abundance, bad weather, and host specificity. These features add variation to the gall landscape and challenge any broad scale hypotheses applicability to a specific area. Currently, there is no consensus as to what explains galling diversity, especially insect gall diversity, but localized hypotheses may be required to yield useful results.

This study aimed to answer, on a local scale, the question of whether soil fertility influences galling insects abundance and diversity. Specifically, I hypothesize less fertile plots will support more galls and gall diversity compared to more fertile plots due to more stressed plants not being able to defend themselves as effectively. Soil fertility in this case simply means higher nutrient amounts (e.g. organic matter, phosphorous, potassium, magnesium, calcium, etc.). Upland and lowland plots were also compared because they are predicted to have different fertility levels. Upland plots should have less water content, organic matter, and nitrogen at a minimum due to how topography and water content effect soil (Lal & Shukla, 2004).

Methods

Location Description, Sampling Period, and Equipment

The location chosen to test the hypothesis was Gabis Arboretum in Northwest Indiana. The climate of Northwest Indiana is temperate continental. The summers and hot and the winters are cold to mild. The average precipitation is near 4 inches a year. The climate is cooler than other areas of same latitude in the Midwest due to the influence of the Great Lakes. This climate data is supported by WeatherSpark.com.

The Gabis Arboretum has a variety of habitats including forest, ponds and steams, savannah, and grassland. Galls are also known to be present and widespread at the arboretum. The surrounding area is rural with scattered forest and tree lines with agricultural crop fields dominating the landscape. The coordinates are 41.448931, -87.153328 (northern hemisphere). Specifically, the habitat type chosen to explore the objectives and hypothesis was grassland. Grasslands have a high density of herbaceous plants which are low to the ground and allow easier inspection than trees. Also, the arboretum's grassland is tallgrass prairie which is known for high floristic diversity and therefore is predicted to have a high diversity of galls.

The study was conducted from late August to late September 2020 (August 25th, September 3rd, September 15th, and September 24th). Several upland and lowland plots (near a stream or pond) were chosen to assess the diversity and abundance of galls (Figure 1). These plots ideally should have different soil nutrient levels and water content. Plots were randomly chosen (not by the author) and approximately 200 square feet areas were measured per plot. Each plot was examined on one day for 40 - 60 minutes for the presence of galls. Plot 1 was searched on August 25th, plot 2 and 3 on September 3rd, plot 4 and 5 on September 15th, and plot 6 on September 24th. Soil was collected on September 24th from all plots and stored in cool temperatures for further analysis.

Equipment required included gall identification books, measuring tape and ruler, camera, plastic bags, notebook, and microscope. The type of microscope used was a light microscope from AmScope and the identification book was Plant Galls and Gallmakers by Felt (1940).

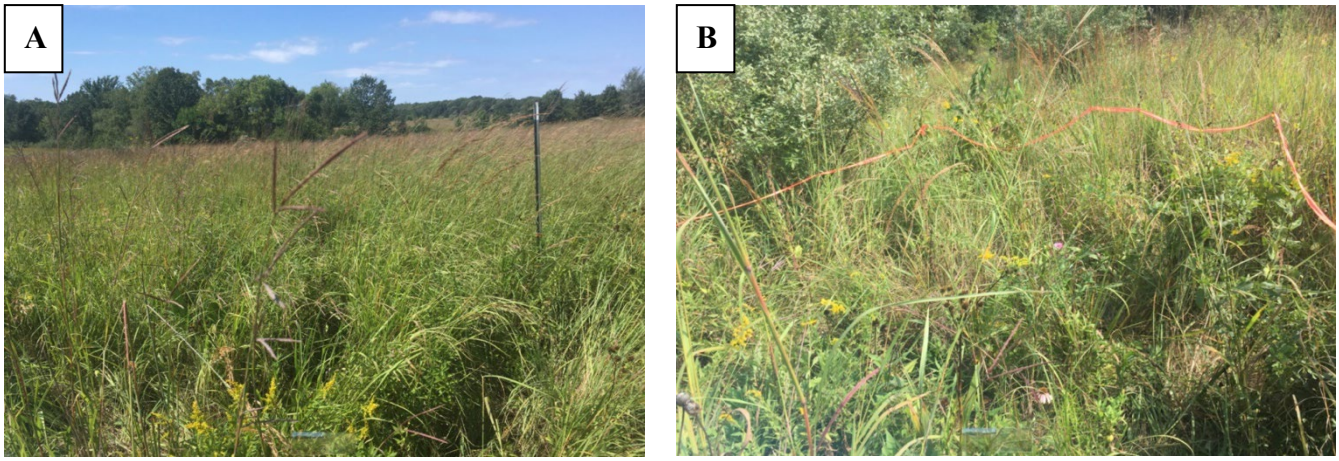

Figure 1: Upland and lowland plots were both rich in plant diversity and density. An example upland plot is to the left (A) and an example lowland plot (B) to the right.

Characterization of Gall Diversity

The diversity and abundance of galls was measured by recording how many gall morphotypes were present in a plot. Plant stems and leaves were scanned by hand for about 40 minutes to an hour for abnormal growths. The plots were searched by starting on one side and moving to the other side to reduce recounts. The author used his background knowledge and experience with galls to sight identify some galls while others required using a guidebook to identify them. Dissection was not usually required but some stems had a similar appearance to galls and were split open to look for a larva or other causal agent to delineate it from normal plant growth. Some galls were identified on site while some were identified at home using photographs. Photographs of each new morphotype were taken. Gall morphotypes were conservatively measured. Any specimen that may have been the same type but was a much different size or if the gall was largely similar to another type and on the same plant then it was not recorded as a new morphotype. Gall abundance was underestimated as well due to roots and seeds not being checked and any internal galls not being detected.

Measurement of Soil Properties

Soil was collected after all the plots were surveyed. Two spots per plot were randomly chosen and about a quart sized plastic bag filled up from each spot. In total, 2 bags per plot with 6 plots means 12 bags of soil were collected. The soil was placed in a cool environment (basement) until cleaned of plant roots and plant material. Then, the soil was sent to A&L Great Lakes for soil analysis. The relevant soil properties measured were organic matter, phosphorous, potassium, magnesium, calcium, soil pH, cation exchange capacity, and nitrate. The fertility of upland plots and lowland plots was determined by averages and statistical difference between upland versus lowland plots.

Statistics

The hypothesis that less fertile plots will have more gall abundance and diversity was tested by comparing the upland and lowland plots gall diversity and abundance using a student's t-test. This statistical test is simple but robust and effective for comparing averages when only two groups are being analyzed. Each plot's two soil sample results were averaged. Then, soil parameters were averaged for the three upland and three lowland plots to compare lowland and upland plots in general. The soil results for upland and lowland were also compared using a student's t-test to determine if the plot types were significantly different. All statistics were determined and analyzed using statistical tests implemented with Excel software.

Results

Gall Diversity and Abundance

The upland plots and lowland plots varied in dominant vegetation. Plots 1,2 and 3 are the upland plots. Plot 1 was flat and dominated by forbs with one large burr oak tree and several young saplings nearby. Plot 2 was mostly flat but on top of a hill and dominated by grass and no trees were present. Plot 3 was also on top of a hill with a couple shrubs nearby and dominated by forbs. Plot 4, 5 and 6 are the lowland plots. Plot 4 and 5 were both about halfway down a hill and dominated by forbs and grasses. Plot 5 had some shrubs. Plot 6 had a high aspect and was adjacent to forest, some shrubs were in the plot while forbs dominated this plot. Overall, Asteraceae dominated the forb community.

Gall abundance was unexpectedly high. Each plot had well over 100 galls present and each plot had Asteromyia galls present in unknown amounts. The Asteroymyia galls are small blister like whitish to reddish galls on goldenrod leaves that were so numerous that they could not all be counted in the defined search intensity time frame. So, a t-test of abundance between upland vs lowland plots will not be useful and not significant as the amount of galls in total was only estimated (stated as hundreds). Bullet burr oak galls were also quite numerous on plot 3. Additionally, gall presence seemed to be controlled by goldenrod and burr oak presence as most galls were found on them. Figure 2 shows examples of some of the most abundant galls. Tables 1 and 2 summarize gall abundance according to morphotype.

Gall diversity was also unexpectedly high. Each plot had at least 3 or more morphotypes. Similar to abundance, gall diversity seemed to be controlled by flora present as goldenrods and burr oaks had the most diversity of galls. A t-test of upland vs lowland plots shows that on average upland and lowland plots had very similar diversity of gall types (p-value = 0.63). Even when total number of unique morphotypes was added together for upland and lowland plots there was 13 unique total morphotypes for lowland and 10 for upland. Overall, 19 morphotypes were

recorded while the author expected around 5 to 10. Some galls were at every plot like the Asteromyia galls and the goldenrod ball gall. Others were only at one plot like the bush gall and poison ivy blister galls. Only 9 of 19 galls were identified to a specific name/what caused it. This does not affect the hypothesis or objectives of the study as the specific name or causal agent was not necessary for this study.

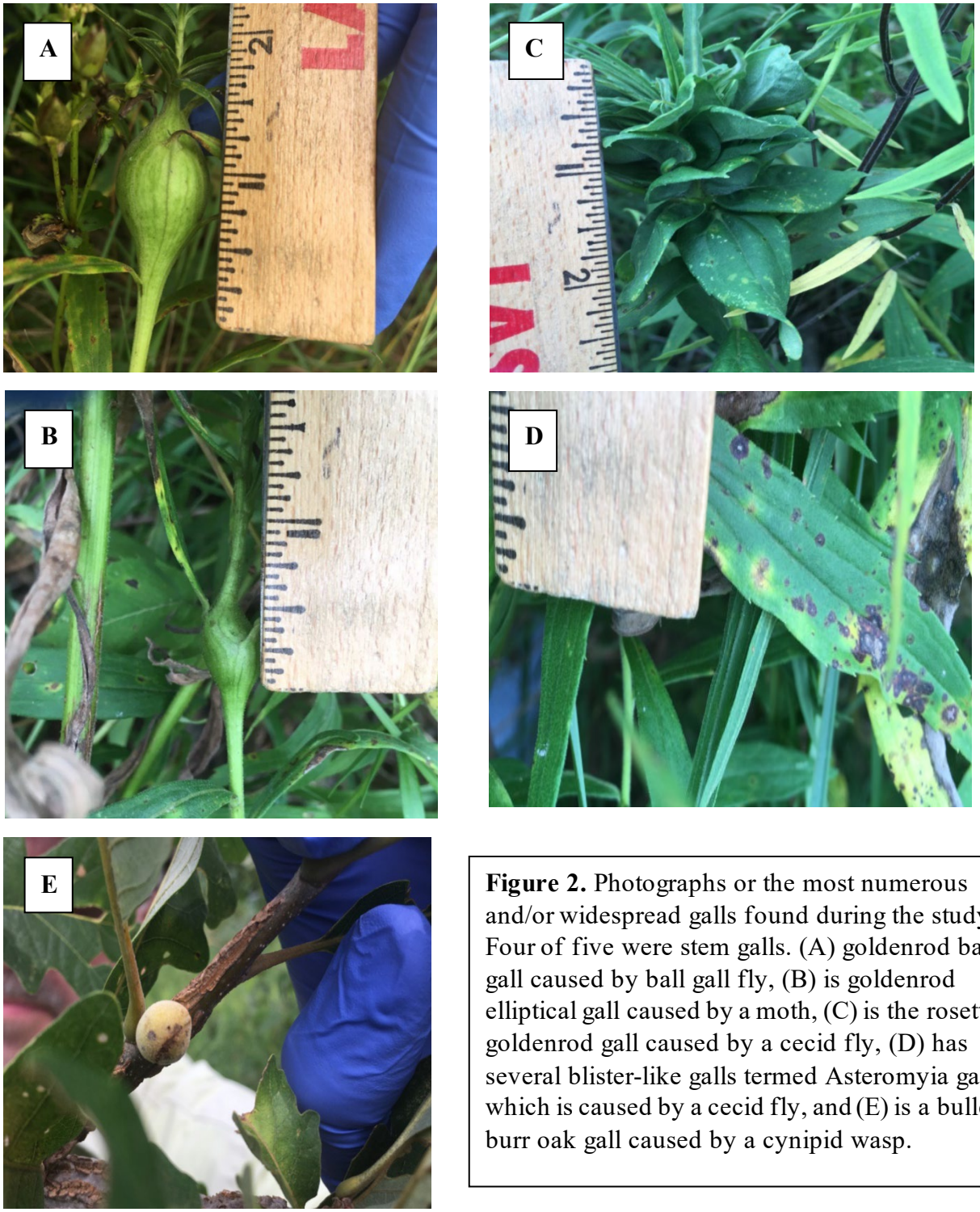

Figure 2. Photographs or the most numerous and/or widespread galls found during the study. Four of five were stem galls. (A) goldenrod ball gall caused by ball gall fly, (B) is goldenrod elliptical gall caused by a moth, (C) is the rosette goldenrod gall caused by a cecid fly, (D) has several blister-like galls termed Asteromyia gall which is caused by a cecid fly, and (E) is a bullet burr oak gall caused by a cynipid wasp.

Upland					
Plot 1		Plot 2		Plot 3	
Morphotype	Abundance	Morphotype	Abundance	Morphotype	Abundance
Asteromyia gall	Hundreds	Asteromyia gall	Hundreds	Asteromyia gall	Hundreds
Goldenrod ball gall	34	Goldenrod ball gall	1	Goldenrod ball gall	4
Goldenrod rosette gall	4	Goldenrod rosette gall	5	Goldenrod Elliptical moth gall	1
Goldenrod Elliptical moth gall	1			Goldenrod nun midge	1
Jewel oak gall	3			Bullet burr oak gall	> 50
Bullet burr oak gall	3			Hedgehog gall	4
Cynipid gall (?)	2			Philonix gall	1
				Cynipd gall (?)	2

Lowland					
Plot 4		Plot 5		Plot 6	
Morphotype	Abundance	Morphotype	Abundance	Morpotype	Abundance
Asteromyia gall	Hundreds	Asteromyia gall	Hundreds	Asteromyia gall	Hundreds
Goldenrod ball gall	6	Goldenrod ball gall	1	Goldenrod ball gall	3
Aster deformed green/brown gall (?)	15	Goldenrod rosette gall	2	Goldenrod rosette gall	7
Aster bulky gall (?)	1	Goldenrod ellipitcal moth gall	1	Bee balm cone galls (?)	5
Lowlying herb gall (?)	2	Goldenrod small head gall (?)	1	Bee balm blister galls (?)	> 10
		Aster deformed green/brown gall (?)	1	Herb bulb gall (?)	1
		Aster bulky gall (?)	5	Poison ivy gall mite blisters (?)	Hundreds
		Bush gall (?)	1		
		Bee balm cone galls (?)	1		

Tables 1 and 2. Summary tables showing morphotype abundance for upland plots (1) and lowland plots (2). Question marks on charts indicate unidentified morphotypes.

Soil Fertility Characterization

The soil nutrient levels, pH, organic matter, and CEC for upland versus lowland were not very different but lowland plots did have higher nutrient levels for every measured nutrient except phosphorous and had higher pH and organic matter. However, there were zero significant differences. However, CEC, pH, calcium, and magnesium were nearly significant with p-values less than 0.1. Nitrate levels were low for all plots and soil pH was near the 5.5 to 6.5 range which is where most soil nutrients are all available for plant uptake (Taiz et al, 2018). Summary of soil analysis results are included in Tables 3-5.

The soil results indicate that the difference between plot types was not as large as desired. This makes the test of the fertility hypothesis less strong and less likely to support or deny the hypothesis. However, the lowland plots were on average more nutrient rich and did have more gall morphotypes which could be due to plant diversity in those plots or due to galling insects at the arboretum benefitting more from vigorous plants instead of stressed plants.

	Soil Nutrient Analysis							
	Upland				Lowland			
Nutrient (ppm)	Plot 1	Plot 2	Plot 3	Average	Plot 4	Plot 5	Plot 6	Average
Phosphorous	29.25	7.5	26.25	21	15	21.25	3	13.0833
Potassium	65	69.5	100.5	78.3333	122.5	103	72.5	99.3333
★ Magnesium	205	170	102.5	159.167	360	380	155	298.333
★ Calcium	875	600	550	675	1175	1150	775	1033.33
Nitrate	1	1	1	1	1.5	1	1	1.16667

	Organic Matter, pH, and CEC							
	Upland				Lowland			
Property	Plot 1	Plot 2	Plot 3	Average	Plot 4	Plot 5	Plot 6	Average
Organic Matter %	5.35	3.9	3.4	4.21667	4.45	4.85	3.5	4.26667
★ pH	5.7	5.6	5.6	5.63333	6.45	6.3	5.85	6.2
★ CEC in meq/100g	8.65	7	5.7	7.11667	10.35	10.95	7.15	9.48333

T-tests	
Organic Matter	0.934014
Phosphorous	0.551012
Potassium	0.496201
Magnesium	0.094622
Calcium	0.067638
Nitrate	0.42265
CEC	0.096732
pH	0.070497

Tables 3-5. Summary of soil nutrient analysis (3), other soil properties (4), and t-test results for soil (5). Stars indicate nearly significant soil results ($p < 0.10$).

Discussion and Conclusions

The purpose of this study was to evaluate soil fertility effects on galling. Galls were examined in upland and lowland plots to accomplish this. The hypotheses and question proposed in the introduction have mixed support. Less fertile plots did not have more galls by plot or in total number of unique morphotypes. Therefore, differences in soil nutrients and overall soil conditions don't appear to be the primary factors that explain gall prevalence across grassland sites on a local scale. An alternate hypothesis to explain these results could be that variation in vegetation (e.g. host plant species and abundance) more strongly determines overall galling abundance and diversity. There is some support for this alternative hypothesis from this study. Most of the galls (90%+) were on goldenrod (Asteromyia is on goldenrod) which is also the dominant forb in the grasslands at Gabis Arboretum or burr oaks (unidentified cynipids were on burr oak), two of only several dozen species of plants encountered in the plots. Also, 11 of 19 gall morphotypes were from burr oak or goldenrod which is 58% of the diversity. Gall abundance was therefore skewed by the dominance of Asteromyia galls, making it difficult to detect differences between plots. Asteromyia galls are caused by a cecid midge, which are very common types of gall makers. The high abundance of goldenrod allowed this insect to thrive.

Gall diversity was not different from upland to lowland plots. This means the null hypothesis is supported. There are several possible explanations for this outcome. One is that the plots were too close together and therefore could have been too similar to detect differences in gall abundance and diversity. However, galling insects are generally poor dispersal agents and the plots were separated by several hundred feet at least. While the galls may be part of the same founding population, they probably did not move from one plot to the next this year which allows any differences in plots to affect the galling outcome. Another explanation is that the soil from one plot to the next also did not vary enough in nutrient or moisture levels. This explanation is supported by the t-tests and soil analysis data, which did not differ significantly across plots. Additionally, soil nutrient levels and moisture content vary over longer time periods, including over month and by seasons. This variation was not accounted for using the sampling methods in this study, therefore additional soils samples and galling surveys are needed over multiple years to potentially capture variation.

The study was small in scale so it inherently has limitations on the conclusions it can draw. Only one area was used instead of several which probably influence the soil analysis and the gall morphotypes present. Only stems and leaves of the grassland plants were examined for galls, despite forests, roots, flowers, and fruits having gall fauna. Also, any early season galls may have been missed but the late season study does capture when most galls will be visible or have already formed. Soil moisture content and nutrient levels should have been measured beforehand as well to ensure different fertility.

In conclusion, plant galls and their prevalence is controlled by many factors. The gall maker, plant, weather, predators, soil, dispersal history, etc. all play roles in determining gall abundance and diversity. This study adds support to the claim that vegetation type controls galls.

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