

The effect of nutrient availability on floral display and pollinator interactions

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Abstract:

Management and restoration are important in helping the ecosystem recover back to an original form. Native plant species and pollinators are a highlight of ecological restoration. Nitrogen is a nutrient source that most plants use to thrive to grow bigger. In high alpine communities, the soil is nitrogen poor and if given treatment can help make native plant communities grow back. We tested whether adding nitrogen affected floral display and pollination. In this study, the field site location is an area that had been stripped away to bare land to build the billy barr community center in Gothic, Colorado. After the billy barr community was successfully built, the land was bare, so there was a restoration project of bringing back native plants in 2017. There are 24 plots were given treatment of either a control, nitrogen in the form of ammonium nitrate, and carbon in the form of sugar. The experiment focused itself in the summer of 2019, to measure floral display and pollinator interactions with the treatments of the plots. The study consisted of counting how many open flowers were open in the plot, the three native plant species that we focused on was *Potentilla pulcherrima*, *Polemonium foliosissimum*, and *Linum lewisii* (flax). I observed pollinators at each plot; where once a pollinator landed on the plant species that was either *Potentilla pulcherrima*, *Polemonium foliosissimum*, or *Linum lewisii* (flax), it would then be noted and what kind of pollinator. The pollinators that we focused on was the native pollinator species in Gothic, Colorado which consisted of *Bombus* spp, *Osmia*, Sweat bees, Mining bees, Bee flies, True fly, Pieridae, Lycaenidae, Swallowtails western tiger, *Papilio gothica*, Checker spots and *Speyeria*. These observations took place several times, to have data to test our hypothesis. We found that nitrogen has no effect on floral display and on pollinator visitation. We did find that having a bigger floral display increased pollinator visitation. A recommendation for restoration is to have large flower patches to increase pollinator visitations.

Introduction:

Disturbances in biological communities are caused by three factors such as, direct human impact, biotic pressure, and environmental changes known as habitual degradation. These factors can cause competitive exclusion which will reduce species richness at low levels of disturbance, and whereas high levels of disturbance exclude all but the most disturbance tolerant species (Mouillot et al. 2013). “Ecological restoration refers to the process of managing or assisting the recovery of an ecosystem that has been degraded, damaged or destroyed as a means of sustaining ecosystem resilience and conserving biodiversity.” (The United Nations Convention on Biological Diversity)

Native species reintroduction has become important in conservation throughout the world for recovery of species that are rare and the purpose of restoration (Godefroid et al. 2011). When

native plants are restored, there is more of an abundance of a native pollinator in the area (Forup et al. 2007).

The mineral needed for a plant's nutrition source is nitrogen. Nitrogen helps a plant become more abundant. Plants have evolved mechanisms to acquire nitrogen thoroughly, because it is common for the soil to be disrupted which can cause nitrogen to be lost. Nitrogen has shown to increase productivity of certain plant species (Pennings et al. 2005). Stimulation of plant growth and photosynthesis has been shown with an increase of a nitrogen supply (Lawlor 1995). When there is an increase in nitrogen, there is an increase in display and corolla size of flora. Insects are most likely to visit plants that have additional nitrogen in the soil rather than plants that have no additional nitrogen. This predicts that pollinators respond to flora with changes in their display and corolla size (Majetic et al. 2017).

Floral display is the number of open flowers a plant will have. Many pollinator visits consist of more open flowers in a patch. Butterflies and bee flies tend to like the open flowers on the canopy of a patch, whereas short tongued bees have a positive relationship with the open flowers near the stem of a plant (Thompson 2001). Bigger floral displays attract pollinators more per unit of time; this can help with cross-pollination. Plant's in larger floral displays may have better fitness (Ohashi et al. 2001). Pollination is required for reproduction in over 80% of the world's plants (Ollerton et al. 2011).

To engage in a successful fruit set, the plant must have functioning reproductive organs. If the reproductive system is functioning normally, then pollination can be viable and successful (Gazit 1976). Pollination has shown to increase fruit set, whether it is by hand pollination or by pollinators themselves such as flies or solitary bees. (Campbell 1987).

The purpose of this study is to determine the nutrient treatment that influences flower number, to determine if floral display influences pollinator visitation, and if treatment of visitation affects fruit set. We hypothesize that when nitrogen is added, the flower number is higher and will create a bigger floral display. We predict that when the floral display is bigger, it will attract more pollinators. When there are more pollinators, it will create a higher reproduction and therefore a higher fruit set. Therefore, to test this, we will have specific methods that will be used to collect the data to analyze our hypothesis.

Methods:

Study Systems

This experiment was conducted in Gothic, Colorado, USA, according to the map on page 8. The latitude and longitude of the field site location is 106° 59' 25.31" W 38° 57' 34.496" N.

In Gothic townsite, near the billy barr community center there are 24 plots that are divided into groups of three. The nutrient addition experiment began in 2017. The treatments were assigned randomly. Each group has three treatments, one plot that is a control, the next is nitrogen in the form of ammonium nitrate was added, and carbon in the form of sugar was added for the last treatment. These treatments are added annually in equal increments during the field season which takes place from May to August. There are 18 plots that lay along a hillside, and the other 6 are located on flat ground in front of the billy barr community center. (Reithel, personal communication).

Floral Display Count

We studied three plant species. *Potentilla pulcherrima*, *Polemonium foliosissimum*, and *Linum lewisii* (flax). These three native plant species were chosen because they were the most common in all 24 plots and were blooming in the time of the start of study.

Once the plants have started to show open flowers, I counted three species of flowers in each plot. The focus of the flower species is *Potentilla pulcherrima*, *Polemonium foliosissimum*, and *Linum lewisii* (flax). To measure floral display, I counted each open flower and recorded it in the data sheets. Once the data was collected, then I will process and analyze the data to determine the variation in floral display. I counted the open flowers each plot every time new data is collected, because some flowers will wilt and then the buds can bloom within a short period of time which affects the flower number count.

Pollination:

I collected data on pollinator observations, using a 10-minute interval method for each plot. I randomly chose the order out of the 8 groups then randomly chose which of the 3 plots I would collect data from first, and then sit close to the plots to where I can identify the pollinators with a naked eye. I then set a 10-minute timer and note the time of day and then start collecting observational data. Once a pollinator lands on the species of flowers, I monitored that specific open flower. I noted it down and the specific pollinator.

Lastly, once all the data has been collected, I analyzed the data using JMP.

I observed a variety of pollinators, as listed starting with bees: *Bombus* spp, *Osmia*, Sweat bees, Mining bees, Bee flies, True fly, Pieridae, Lycaenidae, Swallowtails western tiger, *Papilio gothica*, Checker spots and *Speyeria*. The insect collection at the Rocky Mountain Biological Laboratory was a source of reference of the common pollinators that are local in this region.

Results:

Nutrient Treatment with Flower Number

Nutrient treatment did not have an effect on *Potentilla* flower number ($F=.04$, $p=.96$, Table 1), *Polemonium* flower number ($F=.31$, $p=.74$, Table 2), and *Linum lewisii* flower number ($F=.41$, $p=.67$, Table 3). According to the data tables 1, 2, and 3, the treatment had no significant effect on the flower number.

Nitrogen Treatment with Pollinator Visitation

According to the data results in Figure 1, Figure 2 and Figure 3, nitrogen has no effect on pollinator visitation. *Polemonium* has a $p=0.21$, which shows a trend but it's not significant. Where *Linum lewisii* and *Potentilla* had a p value above 0.50 which makes it not significant.

Floral Display and Pollinator Visitation

According to the data results in Figure 4, the $p=0.0005$ and the $R^2=0.43$. This is significant showing that with more open flower display of all three species, has an increase in pollinator visitation.

Discussion:

We hypothesized that nitrogen is added, the flower number is higher and will create a bigger floral display. This has showed that nitrogen does not increase flower number. This could be from multiple factors such as this was the first year that the native plant species flowered since been given the nitrogen treatment, and if to continue this research then there could be a noticeable difference with the nitrogen treatment added. A second explanation is that nitrogen does not affect flowers in general. For example, that a different nutrient could be more effective. It is shown that having soil with high phosphorus conditions increases final leaf biomass, flower production, and mean pollen production per flower (Poulton et al. 2001). Another reason is that possibly nitrogen has no effect on the plant species that was chosen for this experiment. Other research suggests that nitrogen only affects certain types of plant species (Pennings et al, 2005). Whereas some plant species could show different results to the treatment.

The next hypothesis that we tested was when nitrogen is added, pollinator visitation is more abundant. This experiment has shown that nitrogen does not increase pollinator visitation. Research has found no effects of nitrogen enrichment on the core group of subalpine plants and pollinators or on plant pollinator network structure parameters, such as network topology (Burkle et al. 2009).

The last hypothesis that we tested was when the floral display is bigger, it will attract more pollinators. This shows that having a bigger floral display will attract more pollinators.

There are other researchers that have found that floral display size on the longevity of individual flowers, can vary with pollination rate in many plant species (Harder et al. 2005).

We were not able to complete and test the fruit set of the native plant species for our final hypothesis, since we ran out of time for the field season to conduct the data and analyze it. It was a late snowmelt year which made the plants bolt later in the field season, which would relate the fruit set would be later in September.

Future research could look at the effect of moisture and floral display. During the experiment I observed that the west facing hillside showed to retain moisture better rather than the south facing hill. This could indicate the aspect of where to plant certain native species. Some plant species require more moisture in the soil and I suggest planting those where the sun is not directly on the plot all day. In high alpine locations, water evaporates quicker in direct sunlight. The south facing hill was dry and had less plant growth overall which followed with little to no pollinators.

To focus back to restoration and management on how to help with growing native species, there are ways that nitrogen can be added into the soil for the plants. Nitrogen treatment does increase plant growth (Fahl et al, 2015). Additional nutrients such as phosphorus and potassium could be added since nitrogen did not affect floral display. Finally, to increase native pollinators, having an abundant number of open flowers will help. This can be done by manipulating the area to have large patches of flowers rather than many small patches.

In conclusion, nitrogen does not influence floral display and pollinator visitation. Larger floral displays had more pollinator visitation.

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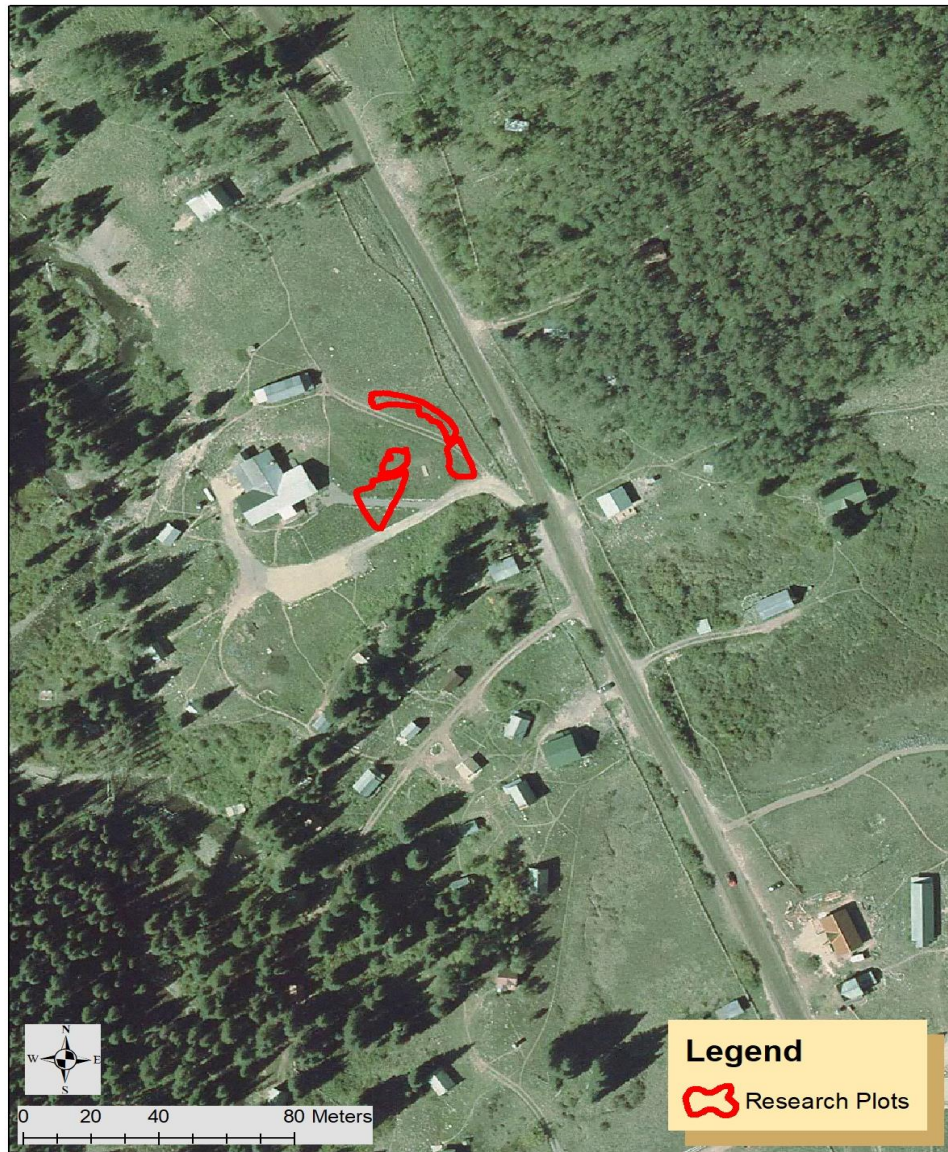
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Map:



Field Site Location: 106° 59' 25.31" W 38° 57' 34.496" N

Potentilla
(Table 1)

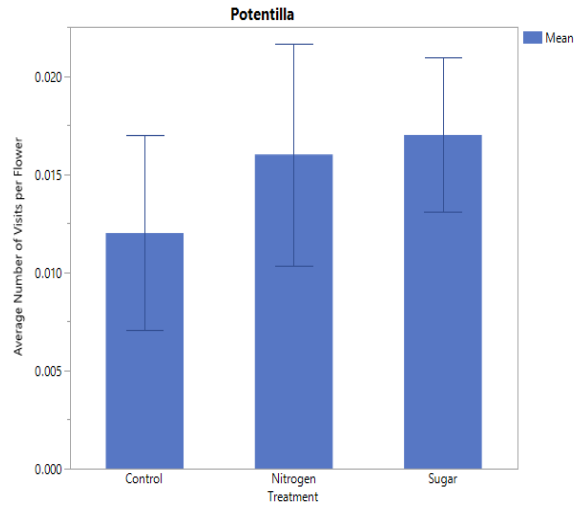
Treatment	Group #	Mean of open flowers	Standard Deviation
Control	8	55	15
Nitrogen	8	54	15
Sugar	8	49	15

Polemonium
(Table 2)

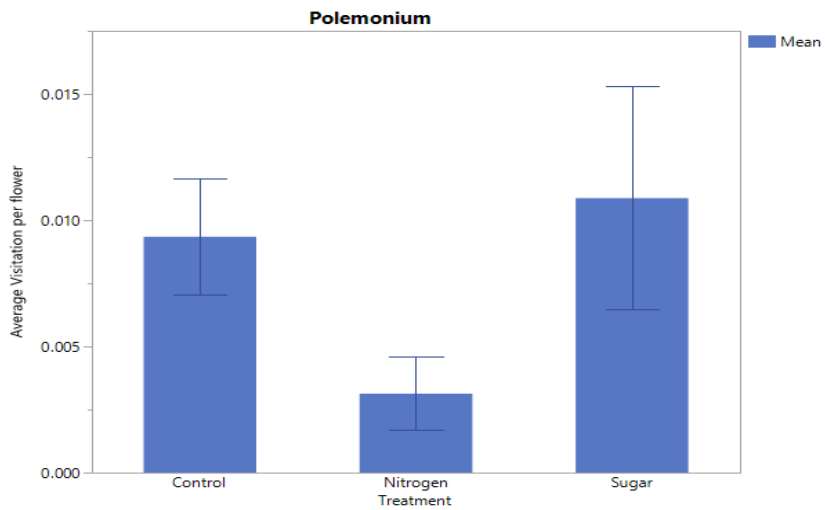
Treatment	Group #	Mean of open flowers	Standard Deviation
Control	8	25	14
Nitrogen	8	36	14
Sugar	8	39	14

Flax (linum)
(Table 3)

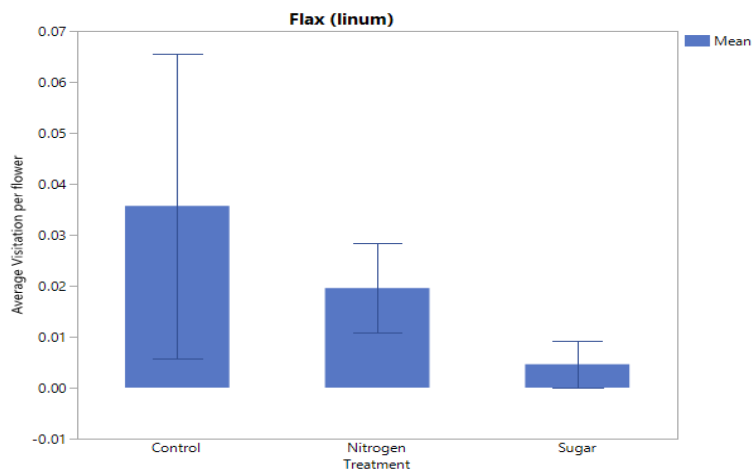
Treatment	Group #	Mean of open flowers	Standard Deviation
Control	8	18	10
Nitrogen	8	15	10
Sugar	8	6	10



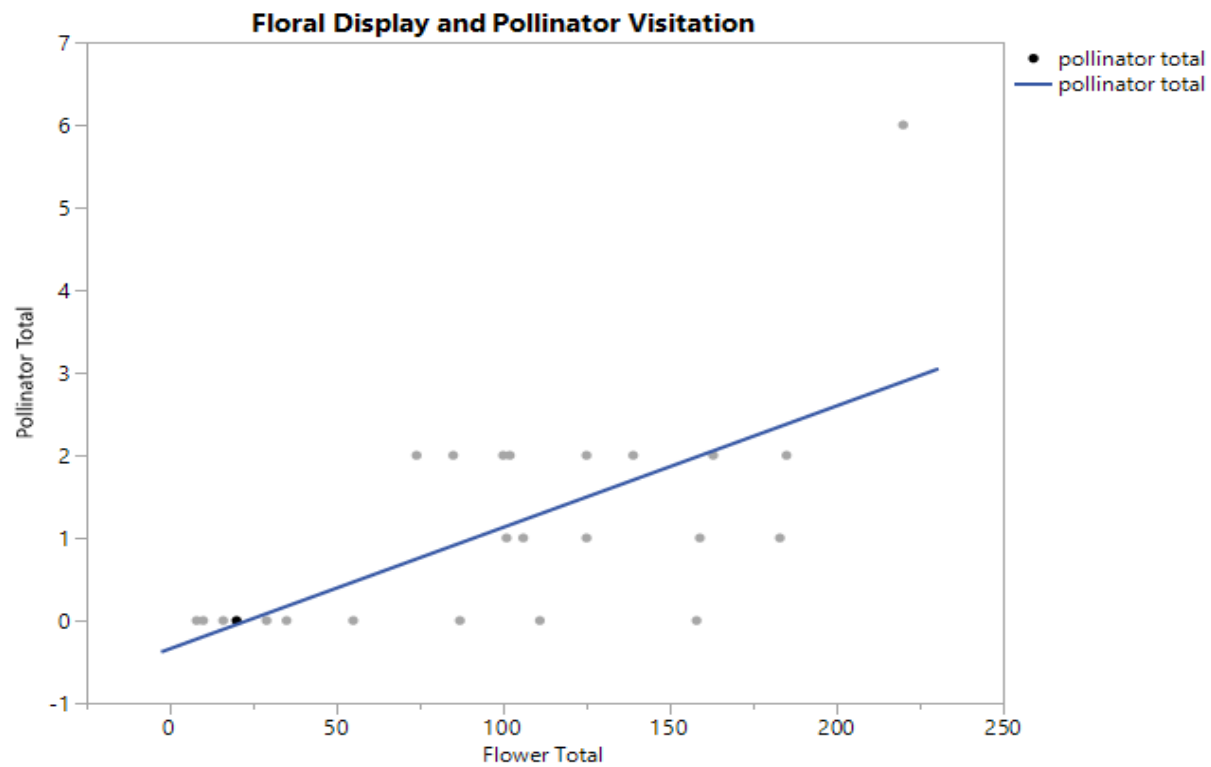
(Figure 1: Nitrogen treatment of potentilla and pollinator visitation)



(Figure 2: Nitrogen treatment of polemonium and pollinator visitation)



(Figure 3: Nutrient treatment of Linum Lewisii and pollinator visitation)



(Figure 4: Floral Display and Pollinator Visitation)