

The Effects of Soil Nitrogen Availability on Plant Reproduction and Solitary Bee Behavior

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Abstract:

Changes in nitrogen availability can alter plant community structure, composition, and abundance as well as higher order interactions. The goal of this study is to investigate the relationships between soil nitrogen, plant reproductive success, and solitary bee behavior and reproduction. Three different nitrogen treatments were applied to plots in subalpine meadows at the Rocky Mountain Biological Laboratory. First, comparisons of female plant fitness among the nitrogen treatments were estimated using stigma pollen receipt in *Campanula rotundiflora*, *Helianthella quinquenervis*, *Lathyrus leucanthus*, and *Vicia americana*. *Lathyrus leucanthus* received significantly more pollen in plots receiving no nitrogen than in those receiving high or low treatments. Second, pollen transport among the plots was estimated using fluorescent dyes. No relationship was found between nitrogen treatment and frequency of dye/pollen transport. Finally, solitary bee nesting boxes were placed in the field to collect information on solitary bee reproduction and behavior, but were used too infrequently by the bees to provide significant data. Further research is needed to better understand plant-pollinator interactions in this nutrient limited system.

INTRODUCTION

Anthropogenic sources of nitrogen are altering the global nitrogen cycle. Between the years of 1961 and 1997, nitrogen inputs in the United States doubled, primarily from fertilizer use and fossil fuel combustion (Fenn et. al. 2003). Nitrogen deposition is of particular concern among the alpine and subalpine vegetation of the Colorado Rockies (Bowman et. al. 1993). During nine months of the year, snow collects nitrogen from urban centers and falls patchily on alpine areas. As the weather warms, snowmelt leaches nitrogen into the soils and streams, resulting in high concentrations in moist meadows and other sensitive areas (Fenn et. al. 2003). These interactions make the impacts of nitrogen deposition a pertinent and significant research subject.

Furthermore, nitrogen is a limiting nutrient in many terrestrial ecosystems (Vitousek et. al. 1997). Changes in nitrogen availability can alter plant community structure, composition, and abundance (Bowman et. al. 1993, Fenn et. al. 2003). These changes can impact nitrogen and carbon cycling, and lead to loss of biodiversity (Vitousek et. al. 1997). Increased nutrient availability can also lead to changes in the reproductive success of plants. Plants may allocate the additional resources directly to enhanced fruit and seed production (Campbell and Halama 1993). Indirectly, the higher quantity and quality of floral rewards produced by nutrient enhancement may result in more pollinator visits (Muñoz et. al. 2005). Alternatively, nitrogen addition can reduce plant fitness, causing sensitive species to become rare or obsolete (Fenn et. al. 2003). Whether plants respond positively or negatively to nitrogen addition, these changes have implications for plant-pollinator relationships.

Plant-pollinator mutualisms can be affected by changes in soil nutrient availability. Nitrogen, in particular, serves as an interesting nutrient to monitor, as it may be passed from plants to their pollinators through amino acids in nectar and proteins in pollen. Bumblebees have been shown to differentiate between high and low quality patches for pollen collection (Robertson et. al. 1999). Some species of butterflies show preference for nectar with high amino acid content, and their fecundity is enhanced by nutrient rich nectar at the larval stage (Mevi-Schutz and Erhardt, 2005). Low levels of nitrogen may limit pollinator fitness, abundance, and distribution (Muñoz et. al. 2004). Changes in nitrogen availability may have consequences at higher trophic levels as well, as it is known to limit primary production in many communities (Bowman et. al. 1993).

The goal of this study is to investigate the relationship between soil nutrients, plant reproductive success, and solitary bee behavior and reproduction. Solitary bees collect both nectar and pollen for their offspring, and their provisions and nests are known to reflect the quality and quantity of resources available to them in a given season (Torchio 1989). Using nitrogen treatments applied to soil patches over two growing seasons, I tracked the use of pollen from solitary bee-pollinated wildflowers exposed to different levels of nitrogen availability. By looking at a measure of female plant reproductive success, I investigated a specific mechanism that is known to cause changes in plant community structure, composition, and abundance in response to increased nitrogen. I also sought to understand how this mechanism relates to pollinator behavior and finally, how the changes in the quantity and quality of floral rewards among flowering plants might alter the reproductive success of their pollinators.

Specifically, this research attempts to answer the following questions. With regards to the reproductive success of plants, 1) Does female plant reproduction vary between different nitrogen treatments? 2) Do the stigmas of flower species pollinated by solitary bees receive different quantities of pollen depending on the nitrogen treatment in which they grow?

With regards to solitary bee behavior, (3) Do solitary bees preferentially visit flowers growing in soil with high nitrogen availability? (4) What flower species do they use to provision their offspring? Concerning solitary bee reproduction, (5) is increased reproduction exhibited by the pollinators that collect pollen/nectar from nitrogen rich plots for their offspring provisions? If so, is increased fitness expressed through larger offspring, more offspring, or altered sex ratio?

MATERIALS AND METHODS

Study System

Research was conducted at the Rocky Mountain Biological Laboratory (RMBL). RMBL is located in the Gunnison Basin of the Rocky Mountains in Central Colorado. Similar ecosystems are known to be nitrogen limited and in danger of the negative effects associated with nitrogen deposition (Bowman et. al. 1993). The growing season extends from late May until early September, while the rest of the year the area experiences extreme snowfall. During the summer months, perennial wildflowers dominate subalpine meadows, and their pollinators breed and nest in close proximity. Research was conducted from late June until early August 2006, during peak production, blooming, and breeding. The foci of this study were the solitary bees, *Megachile sp.*, and seven species of the wildflowers it forages on and pollinates.

Megachilids are abundant pollinators in the subalpine meadows of RMBL. The nesting behaviors and ecology of this family make it an excellent subject for research involving pollen tracing and resource quality. The female nests in long holes found in natural wood or the ground. The large female eggs are laid first, followed by a greater number of small male eggs. The female makes as many as thirty trips to create a provision of nectar and pollen for each offspring to eat when it hatches, 5-6 days after the egg has been laid. The sex ratio and provisions are known to reflect the resources available to the mother Megachilid in a given season (Torchio 1989). Some species hatch out in mid-late summer, while others over-winter in cocoons as larvae or pupae. This variability in emergence timing allows for some identification work to be done in one field season, while also providing opportunity for future data analysis the following season.

Megachilids use a variety of perennial wildflowers to provision their young. I chose seven species to study male and female plant reproduction. *Campanula spp.*, *Helianthella quinquenervis*, *Lathyrus leucanthus*, and *Vicia americana* served as focal species for estimates of female reproduction because of their presence in most plots, receptivity to the dyes amidst rain, and previously documented prevalence in solitary bee provisions.

Nitrogen addition experimental design

I utilized the plots and nitrogen treatments designed by my REU mentor, Laura Burkle. A total of twenty-four 4x4 meter plots have been established among Avalanche Acres, the Copper Creek Trail, and Marriage Meadow on RMBL and Gunnison National Forest land at elevations between 2941-2988 meters. Each site contains at least two sets

of control, low nitrogen, and high nitrogen plots. The low treatment plots receive $1 \text{ g N m}^{-2} \text{ yr}^{-1}$ and have been designed to mimic the estimated quantity of nitrogen deposition on the Front Range of the Rocky Mountains (Sievering et. al 1996). The high treatment plots receive $20 \text{ g N m}^{-2} \text{ yr}^{-1}$. This level of nitrogen applications ensures nitrogen availability to plants even after potential microbial immobilization of the added nitrogen. These dosages were applied in 10 equal treatments of NH_4NO_3 over the course of the summer. All plots were treated in the same manner during the 2005 growing season as well.

Estimates of female plant reproduction

From each plot I collected three stigmas from four focal species of flowering plants, *Campanula rotundiflora*, *Helianthella quinquenervis*, *Lathyrus leucanthus*, and *Vicia americana*. The number of conspecific and heterospecific pollen grains found on each stigma were quantified in order to estimate female plant fitness. These values were compared between the different nitrogen treatments using ANOVA analysis to evaluate the relationship between female fitness and nitrogen availability.

Solitary Bee Behavior

Pollen transport was monitored using fluorescent dye. Dyes have been used to trace pollen and understand pollinator quality among hummingbirds, bumblebees, and solitary bees (Waser and Price 1982; Thomson et. al. 1986; Rademaker et. al. 1997, Adler and Irwin 2006). Using a toothpick, I lightly dusted fluorescent dye onto the anthers of all but three flowers of *Campanula rotundiflora*, *Helianthella quinquenervis*, *Lathyrus leucanthus*, and *Vicia americana* in each plot. Control, low treatment, and high treatment plots received different colored dyes so that the pollen produced under different levels of

nitrogen could be distinguished. These colors were rotated among the different treatments each week to ensure the effects of the nitrogen treatment were not confounded by dye color. The dyes used are non-toxic and should not affect pollinator success I eaten by bee larvae.

The same stigmas used for estimating female plant fitness (see above) were also observed under the microscope for dye particles. Among the different nitrogen treatments, the percent of dye particles of each color present on the stigmas was compared to investigate whether certain plots were used preferentially by pollinators (ANOVA statistical analysis). In all cases, there was equal variance among treatments, ensuring the validity of the ANOVA analyses. The data were also tested for variance. Then, using a dissecting microscope, dye particles were traced to Megachilid nests to look for preferential use of nitrogen treated plots by solitary bees. In order to gain a better understanding of Megachilid pollination behavior, all pollen species present in three miniscule samples of each provision were identified and quantified, also using a dissecting microscope.

Solitary bee reproduction

In each of the twenty-four plots I placed a drilled nest box housing 10-20 removable paper straws in which solitary bees are known to nest (Torchio 1989). The boxes were artificially “weathered” to mimic natural wood, and oriented towards the east at a slight downhill slope to prevent absorption of too much water. Previously, solitary bees have laid their eggs and deposited provisions in such boxes placed in the field around RMBL. Completed or abandoned nesting tubes were removed and made available for further nesting. The completed nests were taken to the lab and the offspring

reared and analyzed. The number of eggs, weight of eggs and provisions, growth rates, and sex ratio for each nest (straw) were compared among nitrogen treatments using ANOVA analysis. All adults that hatched out during the course of the summer were released at the site where their nest was originally collected.

RESULTS

Female Plant Fitness

No significant differences were found in the number of pollen grains received per stigma among the different nitrogen treatments for *Campanula rotundiflora*, *Helianthella quinquenervis*, or *Vicia Americana* (Table 1). In *Vicia Americana*, though the relationship is not statistically significant, the data suggest a strong trend between increased nitrogen and higher number of pollen grains. With higher sample size there is potential for this relationship to be better confirmed.

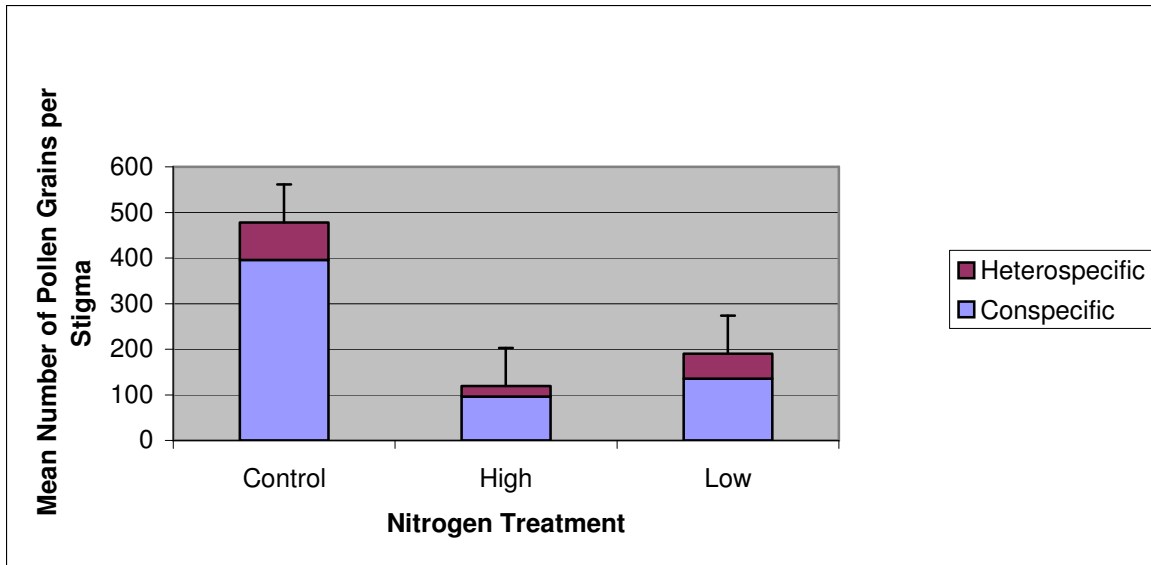
Table 1. Mean number of conspecific pollen grains per stigma.

	<i>Campanula rotundiflora</i>	<i>Helianthella quinquenervis</i>	<i>Lathyrus leucanthus</i>	<i>Vicia americana</i>
Control Treatment	839.306	235.517	395.933	139.567
High Nitrogen	963.486	233.115	96.444	197.439
Low Nitrogen	569.963	274.175	135.500	143.190
F value	$F_{2,7}= .8223$	$F_{2,12}= .0777$	$F_{2,3}= 5.9912$	$F_{2,7}= 1.9481$
P value	.4778	.9257	.0896	.2125
N	10	15	6	18

Lathyrus leucanthus stigmas growing in soil with no nitrogen added were found to receive the highest number of pollen grains (Figure 1). Among heterospecific pollen this relationship was significant ($F_{2,3}=11.0704$, $p=.0412$). While for conspecific and total

grains received, a larger sample size may be needed to validate this relationship ($F_{2,3} = 5.9912$, $p = .0896$; $F_{2,3} = 6.5917$, $p = .0798$, respectively).

Figure 1. The stigmas of *Lathyrus leucanthus* growing in plots receiving no nitrogen additions received significantly more pollen than those with high or low nitrogen treatments.



Solitary Bee Behavior

The dye/pollen transport data show that flowers growing in control, low, and high nitrogen plots receive significantly more pollen from their own plots than from other plots (Table 2). Low treatment plots appear to receive the most pollen from other plots and have the highest standard deviations.

Table 2. Average percent dye contribution from the three nitrogen treatments

Source Plot N-Treatment	Mean % dye contribution			$F_{2,12}$	P value	N
	Control	High	Low			
Control	69.0304	5.4954	25.4741	4.4905	.0350	15
High	17.9487	67.7070	14.3443	13.0264	.0010	15
Low	27.6852	2.7778	69.5370	3.2607	.0740	15

Solitary Bee Reproduction

During the 7 weeks of this research, only two full tubes were collected from the 24 nesting boxes set out for study of solitary bee reproduction. This extremely low sample size does not provide enough data for statistical analysis of the relationship between solitary bee reproduction and soil nitrogen availability. This experience does, however, provide several important lessons for future study of solitary bees in the Gunnison Basin. Nesting boxes in all 24 plots were placed directly on the ground, where they were subject to water-logging and ant infestations that likely prevented solitary bee nesting. Future studies should consider placing the boxes on existing natural nest sites, like logs or stumps, to avoid these problems and increase nest visibility. In addition, solitary bees greatly prefer the non-waxy cardboard and replaceable paper liners produced by Knox Cellars Native Bee Pollinators®, which were unavailable during the first half of this study.

At two sites, Avalanche Acres and Marriage Meadow, nesting boxes were attached to logs outside the plots. These boxes were used prolifically and provided valuable anecdotal information about solitary bee nesting behavior and reproduction. Combined with data collected during the 2005 field season, information from the provisions found in these nests was used to determine which plants to use for the female plant fitness component of this study. Further observational study of these nests will provide rich natural history information on solitary bees in the area, little of which currently exists.

DISCUSSION

Female plant fitness

A negative relationship exists between *Lathyrus leucanthus* pollen receipt and nitrogen addition. *Lathyrus Leucanthus* growing in soil receiving no nitrogen addition received significantly more pollen than those growing in soil receiving high or low nitrogen treatments. This relationship can likely be explained by the *Fabacea* family's ability to fix nitrogen from its environment. In conditions of increased soil nitrogen availability, this adaptation may lose its competitive advantage and lead to depressed reproductive success. This and similar species should be monitored in areas of known nitrogen deposition, where loss of diversity is known to occur to similarly susceptible plant communities.

Contrary to the relationship exhibited by its *Fabaceae* family member, *Vicia americana* seemed to respond positively to increased nitrogen. While these data were not significant, an opposite trend was observed and deserves further investigation. Small sample size is a limiting factor to interpretation of the stigma pollen receipt data of all four species of wildflowers. Another field season worth of data collection may help strengthen the validity of these results.

Solitary bee behavior

These data suggest there is no preferential use of flowers from any one of the nitrogen treatments. In every treatment, stigmas of all four wildflower species studied receive significantly more pollen from flowers growing in their same treatment. This study has been, however, limited to only four wildflower species and there is potential that this trend does not hold true for the entire community.

Conclusions

While the patterns observed for *Lathyrus leucanthus* (and potentially other flower species) provide interesting results, more research is undoubtedly needed for better understanding this system. In order to actually achieve the solitary bee reproduction goals of this research, at least another season of observation and experimentation with nesting boxes is warranted. Additionally, larger sample sizes would grant greater statistical power and possibly expose important aspects of the relationship between female plant fitness and nitrogen addition. Finally, explorations into the effects of soil nitrogen on male plant fitness would contribute valuable insight into the nature of plant-pollinator interactions in the context of nitrogen variability.

Anthropogenic nitrogen deposition is altering ecosystems throughout the world (Fenn et. al. 2003, Vitousek et. al. 1997). Sensitive communities, like those of the subalpine meadows of the Rocky Mountains, may be especially susceptible to its effects. Researching how different nitrogen levels affect plant-pollinator interactions will provide valuable insight into the consequences of altered nitrogen cycles. It will also enhance our knowledge of the effects of natural variation of nitrogen availability across a landscape. This research will provide a better understanding of nitrogen limitation on pollinator fitness, abundance, distribution, and potential impacts on other trophic levels. It will also broaden our knowledge of specific plant-pollinator interactions in the Gunnison Basin.

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