How does long-term, low level nitrogen addition affect plant floral traits?

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Abstract

Due to the rapid creation and distribution of synthetic fertilizers, the amount of reactive nitrogen entering the atmosphere has significantly increased, leading to larger amounts of nitrogen deposition across the earth. Nitrogen deposition is an important problem, and a significant driver of global change, yet its impacts on plant-pollinator communities are largely unknown. These communities are exceptionally valuable for their ecosystem services, with pollination services being valued at billions of dollars per year, and also serve to stabilize their greater ecosystems. In spite of this importance, little is known about the impacts of long term, low level nitrogen addition on them. Here, we focused on the impacts of long term nitrogen addition on plant floral traits in four common forbs at a long term nitrogen addition experiment in Almont Triangle State Wildlife Area in montane southeastern Colorado. For this study we measured plant height, plant width and flower number, and used Mann Whitney U tests to assess the impact of the treatments on these traits. We found that there was no significant difference in individual plant flower production in nitrogen and ambient plots. However, *Eriogonum umbellatum* plants in nitrogen treated plots were significantly taller than *Eriogonum umbellatum* plants in ambient plots. All other plants had non-significant effects on plant height between nitrogen and ambient heights. *Eriogonum umbellatum* flowers in nitrogen treated plots were significantly larger than ones in ambient plots. *Eremogone congesta*, *Castilleja linariifolia* and *Achillea* millefolium showed no significant difference between plant height or flower size in the ambient and nitrogen treatment plots. Future directions could include looking at other floral traits such as nectar length, flower length and coriolis diameter, and taking into account soil moisture and year-t–year monitoring of drought conditions in Almont Triangle State Wildlife Area.

Introduction

Anthropogenic changes are both direct and indirect environmental and ecological alterations caused by humans, with significant effects on biodiversity and ecosystem functioning. For example, the rapid increase of greenhouse gasses within our atmosphere causes warming temperatures (Ramanathan, V., & Feng, Y. 2009). Deforestation, urbanization and intensified agriculture expansion significantly alter the physical landscape and often destroy natural habitat. At the same time, synthetic nitrogen fertilizers have enabled highly efficient global crop production, but have significantly disrupted the nitrogen cycle (Qiao, C *et al.* 2014).

Nitrogen is an essential component of biological and ecological processes. Nitrogen can greatly affect biodiversity of plant communities, restructure communities of previously nitrogen-

limited plants and change both community and individual plant functioning (Burkle, L. A., & Irwin, R. E. 2010). The effects of nitrogen on trends such as soil eutrophication and acidification have not been broadly studied, but the increase of global nitrogen deposition in soils is recognized as a threat to biodiversity (David, T. I., Storkey, J., & Stevens, C. J. 2019).

One of the largest groups contributing to biodiversity across the world are insects (Eli, X., & Wiens, J. J. 2023). Within this group, pollinators such as bees, butterflies, flies and beetles carry the task of pollination. Pollination services are valued at billions of dollars per year, and some 90% of flowering plants depend on an animal vector for reproduction (Tong, Z.-Y *et al. 2023*). Pollinating insects rely on flowering plants for food resources; thus, flowering phenology, floral production, and other floral traits are vitally important factors to consider when it comes to understanding the outcomes for plant-pollinator communities under environmental change..

The effects of long-term nitrogen deposition on plant-pollinator communities is still largely unknown, since much past work has focused on either particular species, or short-term, relatively high levels of nitrogen deposition (David, T. I., Storkey, J., & Stevens, C. J. 2019). Nonetheless, there is evidence that nitrogen addition can increase flower size (Shoram, S. *et al.* 2012) in some species, increase flower production (Burkle, L. A., & Irwin, R. E. 2010), and impact flowering phenology (Zhang, T. *et al.* 2026). These, and other environmental impacts, such as earlier flowering or less overall flowering plants can have large consequences for pollinators (David, Storkey & Stevens 2019).

Here, we will leverage a long-term, low-level nitrogen addition experiment (Grinath, J. B. 2018) to elucidate these effects on floral traits at the community scale. The slow-release ammonium nitrate fertilizer randomly placed at half the sites mimics nitrogen rates common in the Western US (Greaver et al., 2012).

At these sites in the Almont Triangle State Wildlife Area, we studied 36 different plots to count flowering plants, estimate cover within each plot, and measure various floral traits to determine how the nitrogen treatment has impacted plant-pollinator communities, specifically plant floral traits. We predicted that the nitrogen treatment would increase species flower size at the plot scale, increase floral production, and increase plant height.

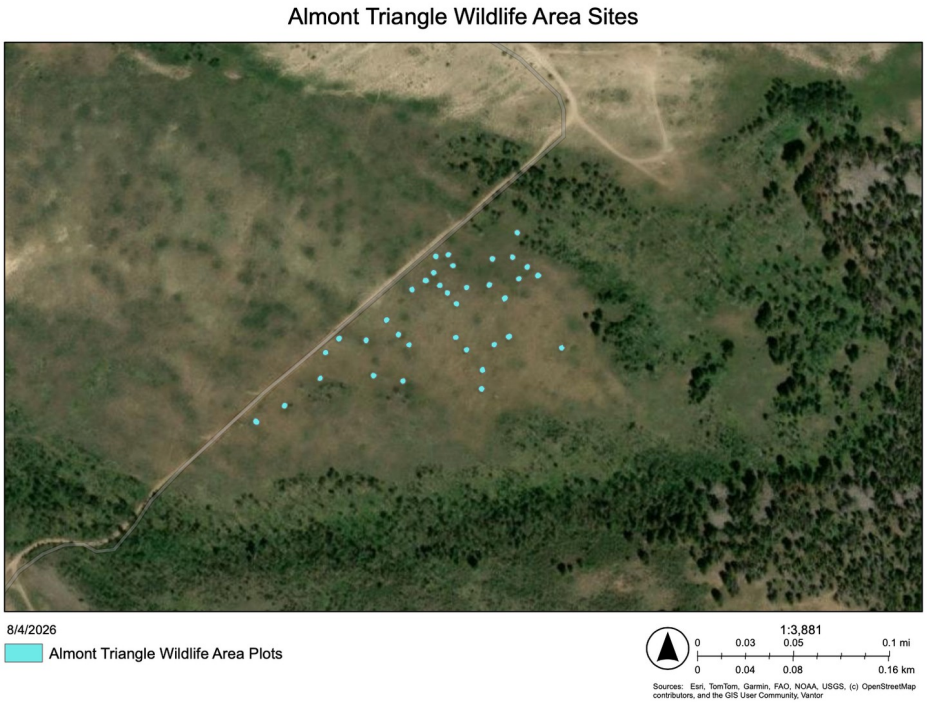

Figure 1: Geospatial map of Almont Triangle Wildlife Area plots.

Methods

At least once a week we surveyed all flowering plants within the 36 experimental plots at Josh Grinath's long-term nitrogen experiment at the Almont Triangle (Grinath, J. B. 2018). This survey included: counting and identifying all flowers to species, measuring all flowers for size with a digital caliper (mm). Flowers that were round and actinomorphic (eg. asters) were measured once for diameters and species that had bilaterally symmetrical flowers (eg. Lupinus sp.) had their diameters measured twice. Flowers that were large enough to reliably measure petal traits had a randomly selected petal measured for width and length. Flowers that were large enough to reliably measure corolla diameter had their corolla diameter measured.

All plants with at least one open flower were measured, and the height of the highest flower was recorded. The height of the plant was also measured.

We used Mann-Whitney U tests in the package *stats* to statistically assess the impact of the treatment on three traits (plant height, floral width and flower production) in the four most common species sampled (*Eremogone congesta* "ARCO5", *Castilleja linariifolia* "CALI", *Eriogonum umbellatum* "ERUM", and *Achillea millefolium* "ACMI2") . The timeline of this research was between June and August of 2026. Data collection was primarily throughout the

months of June and July with early August being used as time for data analysis, paper creation and writing.

Results

We measured 131 ARCO5 flowers, 123 CALI flowers, 876 CHRYS flowers, and 148 ERUM flowers in the 36 experimental plots. Our statistical analyses indicated no significant difference in individual flower production (Figure 2), pooled across experimental treatments in any of the four flower species

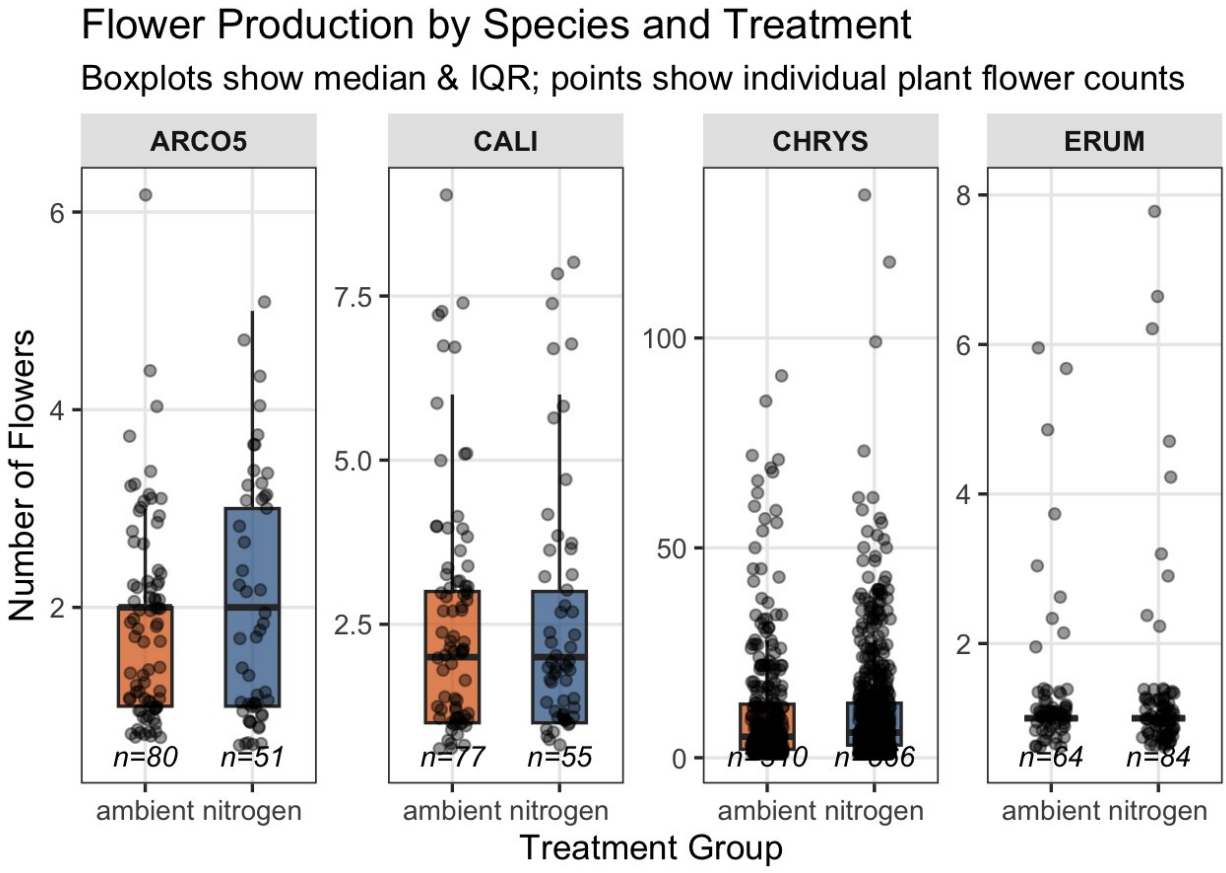

Figure 2: Flower Production by species and treatment for the four most common species sampled, showing both ambient and nitrogen treatments. Between ambient and nitrogen flowers there is no significant difference between the number of flowers produced.

We measured 85 ARCO5 plants, 107 CALI plants, 281 CHRYS plants and 146 ERUM. We found no significant difference in plant height for ARCO5, CALIR or CHRYS (Figure 3); however, ERUM grew significantly taller in nitrogen-treated plots than ambient plots with a significant p value of <0.0001.

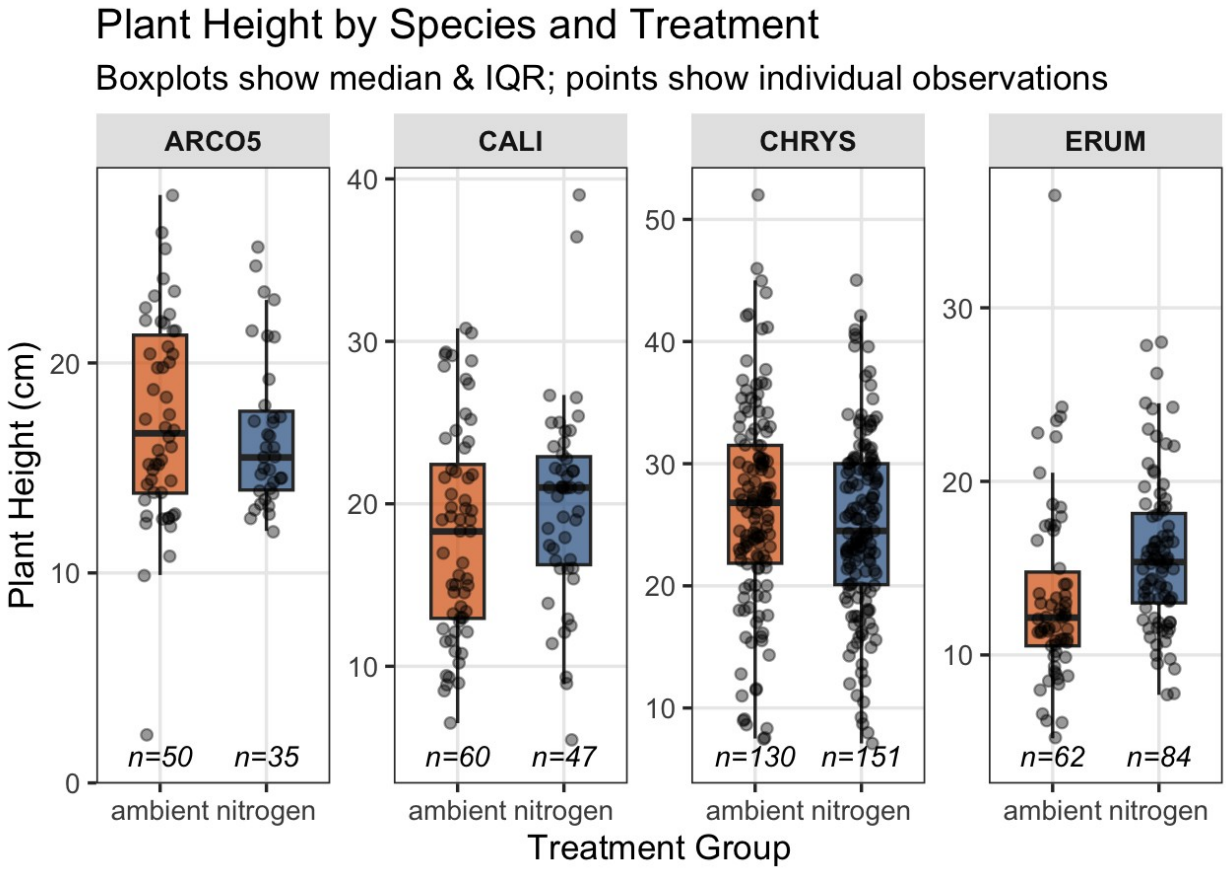

Figure 3: Plant Height by species and treatment for the four most common species sampled, showing both ambient and nitrogen treatments. ERUM plants in nitrogen treated plots were significantly taller than ERUM plants in ambient plots. ARCO5, CALI, and CHRYS had non-significant changes in height between nitrogen and ambient plots.

We measured 135 ARCO5 flowers, 105 CALI flowers, 279 CHRYS flowers, and 144 ERUM flowers in the 36 experimental plots. Our statistical analyses indicated no significant difference in individual flower width across three species, pooled across experimental treatments in any of the four flower species (Figure 4). However, in the case of ERUM, flowers in nitrogen-treated plots were significantly wider than flowers in ambient plots with a significant p value of <0.0001.

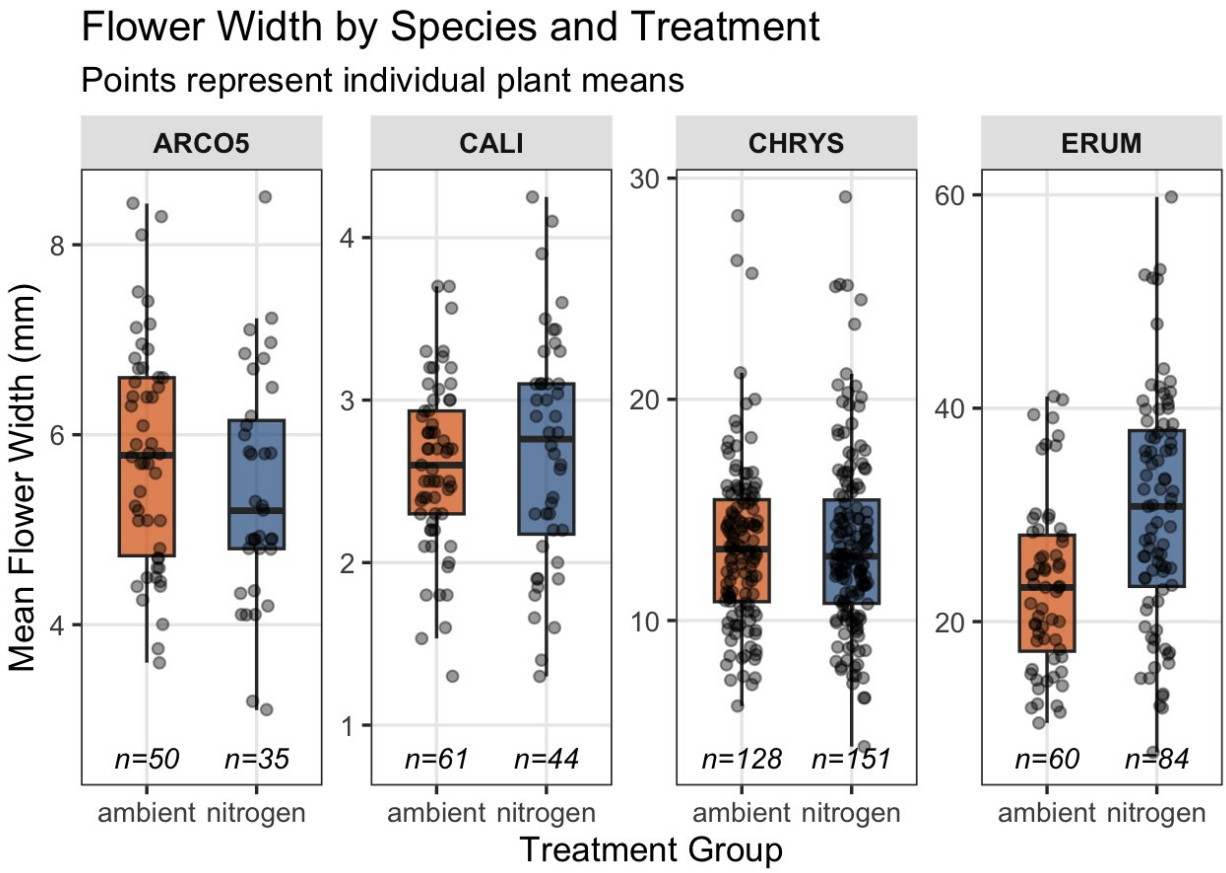

Figure 4: Floral Width by species and treatment for the four most common species sampled, showing both ambient and nitrogen treatments. ERUM flowers in nitrogen treated plots were significantly larger than ERUM flowers in ambient plots. ARCO5, CALI and CHRYS had non-significant changes between nitrogen and ambient flower width.

Discussion

Between the three traits (flower production, plant height and flower width) there was no significant difference found between ambient and nitrogen treated for three of the four most common species (ARCO5, CALI and CHRYS). ERUM showed a significant difference in plant height and flower width between the ambient and nitrogen treatment. Others have found an effect of nitrogen on the traits of some species, whereas other species are unchanged. A similar nitrogen addition study in the Tibetan Plateau showed that nitrogen addition differentially

affected plant growth; in both number species and individuals of forbs, whereas grasses and sedges remained at stable numbers (Zhang, T. *et al.* 2026). This is significant because it shows the difference in relationship between higher altitude study sites and nitrogen, even in places across the world.

Some reasons why we might have seen this species' specific response to nitrogen could include the strange and meager snowpack that Colorado received in the 2025-2026 winter season (Center, C. 2026). This significant decrease of snowpack increased substantial drought conditions in which plants likely responded differently. One of the most common and visible observations seen when flowering species are faced with drought is the failure of flowers to open or to produce flowers in the first place (Su, Z *et al.* 2013, N. Dabagia, *personal observation*).

ERUM might not be as limited (to some extent) by intense drought conditions and therefore was able to utilize nitrogen addition to grow taller and produce larger flowers. This could be explained by Liebig's Law of the minimum which states that optimal growth and performance in biological systems are limited by the least available essential input (Hooker, 1917). In this case, we might assume that under prevailing interseason drought, water was the least available essential input in our experimental plots, and the other three species would have needed more water to reap the effects the added nitrogen induced in ERUM.

Additionally in a model created for seed-sourcing sulfur-flower buckwheat in North American sagebrush steppe ecosystems researchers found a correlation between strongly linked seed source climates at source locations with plant traits evaluated in the common garden, suggesting climate-driven adaptive evolution (Johnson, R. C., 2023).

It is incredibly hard to predict responses to global change at the landscape or regional scale, so long term experiments like this are important to understanding our changing planet, but finding the change with ERUM in our nitrogen plots is a step to better understanding the long-term effects of nitrogen deposition.

Future directions could include more comprehensive analysis of the other traits that we measured such as various other floral measures, computing community-weighted trait means that take into account species abundance,and the incorporation of various climatic and abiotic variables into modeling, such as soil moisture, relative humidity, or air temperature. Additionally, repeating this study over some years can help us better understand the interyear variability of nitrogen impacts on these communities.

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