

Floral phenotypic response of *Ipomopsis aggregata* and related hybrids to changing soil moisture and nitrogen enrichment

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ABSTRACT

Plants are products of their environment. This is also true for flowers, which can be shaped through abiotic factors that lead to plastic response or natural selection. Both floral morphological traits and volatile organic compounds (VOCs) have the potential to be impacted by environmental conditions. Floral morphology has long been understood to shape pollinator-plant interactions. VOCs emitted by floral tissues compose a plant's floral scent, which is important for a variety of reproductive-related interactions. Namely, both the concentration and type of VOCs influence the activity of associated pollinators and herbivores. Learning more about floral morphology and VOCs and their response to abiotic conditions is essential due to their significance to plant reproduction and the current trends in global change. **To contribute to this growing collection of knowledge, we investigated the effects of differing soil moisture conditions and nitrogen enrichment on the floral traits and VOC profiles of the well-studied *Ipomopsis aggregata* and related hybrids.** To achieve this, we performed two experiments. The first was an observational study which monitored *in situ* soil moisture and attempted to correlate that to the emissions of compound. The second experiment was a manipulative study where nitrogen fertilizer was added to understand the effects of increased nitrogen on floral traits and VOCs. In response to nitrogen and water treatments we observed increased corolla width within the hybrid population. In terms of VOCs, the main effect was a larger increase in emission of alpha-pinene and beta-myrcene at night when nitrogen was applied, an effect seen only in the *I. aggregata* population. We did not detect associations of the floral traits with soil moisture. Understanding the effect of environmental conditions on floral traits is essential if we wish to know more about the future of plant-related biotic interactions under climate change.

INTRODUCTION

Floral traits have long been considered to largely be the product of selection exerted by pollinators (Darwin 1862; Harder and Johnson 2009). Given the importance of pollinator mutualisms to fertilization and resulting seed production, this approach to understanding floral phenotypes is well supported. However, it overlooks the potential influence of abiotic factors in determining floral phenotypes (Strauss and Whittall 2006). Factors of a plant's nonliving environment influence much of its physiology, which in turn may influence floral traits such as corolla morphology. Climate and nutrient availability have been shown to alter specific floral characteristics (Caruso et al. 2005; Nattero et al. 2011). As such, abiotic factors have been shown to elicit plastic floral phenotypic responses (Powers et al. 2022) and exert natural selection on floral traits (Campbell and Powers 2015). Alterations in floral traits driven by abiotic factors could then result in consequences for related biotic interactions. In the face of global climate change, it is imperative that we understand the potential for abiotic factors to shape floral phenotypic expression.

In addition to morphological floral traits, floral scents may be shaped by abiotic conditions. Despite often being considered noncommunicative sessile organisms, plants are regularly interacting with their biotic environment. Plants engage in continual interactions via the emission of volatile organic compounds (VOCs), or organic compounds that vaporize under standard environmental conditions (Yuan et al. 2009; Bouwmeester et al. 2019). VOCs are highly diverse, arising from various biochemical pathways across the entirety of a plant. As a result, volatiles have become a valuable tool for plants to communicate information regarding rewards, deterrents, and danger to surrounding organisms. Floral scent is due to VOCs, which can include a wide profile of compounds (Raguso 2008). As a result of dictating pollinator relationships, floral VOCs have extensive influence over which pollen comes into contact with stigmas and leads to reproduction (Chapurlat et al. 2019). VOCs simultaneously interact with herbivores, who can be deterred or attracted through olfactory senses (Campbell et al. 2002). These fitness implications of floral VOCs make them highly attractive as an emerging topic for the study of plant population and community dynamics.

An ideal study system for investigating the effect of environmental characteristics on floral traits and VOC dynamics is the system of *Ipomopsis aggregata*, *I. tenuituba*, and their hybrids. All three groups are monocarpic, perennial, herbaceous plants that grow in montane to sub-alpine conditions. Near our study sites at Poverty Gulch in Gunnison County, CO, *I. aggregata* typically inhabits lower elevation environments while *I. tenuituba* can be found at higher elevations. Between the locations of these populations, they occasionally hybridize and have produced viable advanced generation hybrid populations that inhabit intermediate elevations (Wu et al. 2005). The elevational gradient these populations inhabit show natural variation in environmental conditions. The lower *I. aggregata* site is on average cool and wet while the hybrid and *I. tenuituba* sites, despite being higher in elevation, are comparatively arid and warm due to factors such as aspect and soil type. (Wu and Campbell 2007). Based on previous pollinator observation studies, the red, wide, tubular corollas of *I. aggregata* attract hummingbirds as the primary pollinator while the narrower tubular corollas of *I. tenuituba* appear to promote moth pollination (Campbell et al. 1997). Other morphological traits of *Ipomopsis*, including corolla length, corolla width, style length, anther position, and sepal width, have been shown to be important for a variety biotic interactions. Specifically, these aspects can affect pollinator attraction, pollen transfer, and defense against seed predation (Melendez-Ackerman et al. 1997; Campbell et al. 1997; Campbell et al. 2022). Previous studies on the floral VOC profiles of each *Ipomopsis* species and hybrids have characterized the general composition of each population's floral scent (Bischoff et al. 2014). The most prevalent and significant compounds within these floral scent profiles have also been identified. Notably, only hybrids and *I. tenuituba* emit indole, a nitrogenous VOC known to play a role in hawkmoth pollinator attraction as a potential antennal stimulant (Bischoff et al. 2015). Emission of indole and other VOCs were also found to change depending on the time of day or night, suggesting a function in temporally based biotic interactions. Both indole and the major volatile of alpha-pinene influence seed production and therefore individual fitness (Campbell et al. 2022).

VOCs and floral morphological traits are predicted to respond to the abiotic consequences of anthropogenic climate change (Yuan et al. 2009). In sub-alpine ecosystems, like that of *Ipomopsis aggregata* and related hybrids habitats, changes in nitrogen deposition, precipitation regimes, and snowmelt date are likely to alter soil conditions (IPCC 2021). Specifically, these factors could result in changes to nutrient content and soil moisture

throughout the growing season. In a study measuring VOC response to a lab-controlled drought scenario, *Ipomopsis* individuals showed an overall increase in emissions, but a nonlinear trend of specific compounds, as soil was continuously dried (Campbell et al. 2018). While very few studies have investigated the effects of nutrient fertilizer on floral VOCs (Friberg et al. 2017; Luizzi et al. 2021), no study has ever discussed the effects of nitrogen additions on *Ipomopsis* VOCs. However, there is evidence that increased access to nitrogen promotes growth and carbon assimilation (Campbell et al. 2010). Additionally, Burkle and Irwin (2009) discovered that nutrient fertilization promoted a few floral characteristics of *Ipomopsis* such as corolla width and nectar production but did not measure floral scent. These studies demonstrate the presence of floral phenotypic plasticity in *Ipomopsis*, making the consideration of plastic response in floral traits and VOCs to abiotic conditions even more compelling.

Considering the potential for profound environmental change and the importance of floral traits and VOCs on plant-pollinator interactions, we undertook a study composed of two experiments. They were designed in tandem to answer the question: How do soil moisture or nitrogen influence the phenotypic expression of floral traits and volatiles in *Ipomopsis aggregata* and related hybrids? There were three main hypotheses associated with this project. First, given the findings of previous studies, we expected plants with greater access to nitrogen would be more productive, allowing increased allotment to the emission of floral volatiles and development of floral tissues. Second, responses to soil moisture or nitrogen were expected to vary between *Ipomopsis aggregata* and related hybrids. This prediction was attributed to the unique VOC profiles present in either type of plant and the physiological adaptations to the differing environmental conditions which these types of plants experience (Wu and Campbell 2007). Finally, we predicted nitrogenous volatiles, such as indole, would see increased emission in hybrids when additional nitrogen was introduced, which could have important implications for pollinator specificity regarding hawkmoths. For comparison with the volatile responses, we also examined responses of floral morphology and nectar production.

METHODS

I. Experimental Setup

The sites for both the observational and manipulative studies were subalpine meadows located in Poverty Gulch, Gunnison County, CO, USA. Poverty Gulch hosts populations of *Ipomopsis aggregata*, *I. tenuituba*, and their resulting hybrid. *I. aggregata* populations are found at lower sites (2,900 m), *I. tenuituba* populations are located at higher elevation (3,100 m) and hybrids are present throughout elevations between these populations. We were motivated to study populations of both *I. aggregata* and hybrids by the wider range of floral VOCs and more variation in soil moisture conditions.

To understand how soil moisture influences *Ipomopsis* floral traits, we tagged 56 flowering individuals of *Ipomopsis aggregata* from a relatively low elevation population and 34 flowering individuals from the center of the hybrid zone to monitor for soil moisture. Soil moisture was measured as volumetric water content (VWC) at the base of each individual averaged over two measurements. The first measurement was taken early in the summer growing season, followed by a second measurement during floral VOC measurements later in the season. Because the second measurement was taken at the time of volatile collection, we only utilized the second measurement when investigating the effect of soil moisture on VOCs. To investigate the effect of nitrogen enrichment, 20 individuals were randomly selected from the flowering

populations tagged for soil moisture observation. We added liquid fertilizer composed of 2 g of ammonium nitrate (NH₄NO₃) dissolved in 400 mL water to 10 individuals of each nitrogen experiment group. The remaining 10 plants received a control treatment of 400 mL water. The pouring of each treatment was done by hand at a slow rate to mitigate surface runoff. Treatments were applied once a week for five weeks. At the *I. aggregata* site, additions began the week of June 21st, 2022 and concluded the week of July 19th, 2022. At the hybrid site, nitrogen was added weekly between June 28th, 2022 and July 26th, 2022. In total, we had three treatment groups: nitrogen added, water only added, and ambient plants that received no extra resources. At the end of the five weeks, we collected leaf samples from each treated plant to undergo reflectance spectrometry with an Ocean Optics spectrometer and calculate an index correlated with chlorophyll a (Richardson et al. 2001),

$$ChINDI = (R750 - R705) / (R750 + R705),$$

where R750 and R705 indicate reflectance at wavelengths of 750 and 705 nm. Chlorophyll a concentration is typically proportional to leaf nitrogen content (Evans 1989). The purpose of examining chlorophyll content was to understand the extent to which nitrogen was accumulated by the plants receiving fertilizer.

II. Floral Trait Measurements

We examined the response of morphological floral characteristics to differing amounts of soil moisture and nitrogen by measuring corolla length, corolla width, style length, maximum and minimum anther position, sepal width 48 hour nectar production, and nectar concentration. These morphological characteristics have been shown to influence plant fitness in *Ipomopsis* by a variety of mechanisms (Campbell 1989, Campbell et al. 2022) such as pollen delivery, floral visitation, and seed predation. Each trait was measured 1-5 times per plant with measurements taken on July 7th, 14th, 21st, 27th, and August 2nd, 2022.

Corolla length, corolla width, style length, maximum and minimum anther position, and sepal width were all measured using calipers. We selected flowers for measurement if they had progressed into the female floral phase and had developed a receptive stigma. Corolla length was measured as the distance from the base of the calyx up to the opening of the corolla tube. Corolla width was determined from the inside diameter of the corolla tube. Style length was the distance from the base of the calyx to the stigma. Maximum and minimum anther position were determined by measuring the distance of the highest and lowest anther to the base of the calyx. Finally, sepal width was the distance across the base of an individual sepal.

Nectar collections were prepared using folded drinking straws to cover budding flowers. Straws were left on the bud for 48 hours to prevent any loss of nectar to pollinator or nectar robber visits. At the end of the 48 hour period, 32mm long, 5 µL microcapillary tubes were used to extract nectar from the flower. Measurement of nectar volume was accomplished through measuring the millimeter length of the microcapillary tube filled with nectar. This number was then multiplied by 5/32 to convert length measurements into volume of nectar produced. Values were then divided by 2 to report nectar production on a daily basis. To understand nectar concentration, a refractometer that measures the sugar concentration in nectar was employed.

III. Floral Volatile Sampling

Floral VOC sampling occurred on July 21st, 2022 at the *I. aggregata* site and on July 27th and 28th at the hybrid site. To investigate the VOCs emitted by flowers in response to the different soil conditions present in the study, floral headspace was collected from each plant using dynamic headspace extraction methods (Campbell et al. 2018). An individual flower was covered by an oven bag (7 x 10 cm), allowing the volatiles to equilibrate within the bag for 30 minutes. Headspace was captured from a single flower to reduce contamination from additional plant VOCs not emitted by floral organs. After allowing for the volatiles to equilibrate, a scent trap consisting of a capillary tube with 5 mg of Tenax TA sorbent and glass wool was then connected to a micro air sampler (Supelco PAS-500) pump and inserted into the bag. Air was pumped through the scent trap at a constant rate of 100 mL min⁻¹ for 15 minutes, allowing for the continuous absorption of emitted volatiles. Control samples of ambient air were collected from empty oven bags at the same time and location of floral volatile collection. Ambient control samples underwent an identical process for volatile absorption using scent traps. Sample collections occurred both during the day (9:00 am to 12:00 pm) and at night (8:00 pm to 12:00 am) with the aim of understanding temporal patterns in VOCs.

Volatile samples underwent analysis using thermal desorption gas chromatography – mass spectrometry (GC-MS). Scent trap samples were loaded into a Markes Ultra autoloading system connected to a Markes Unity-xr thermal desorber, which fed volatiles into a Shimadzu GC-MS QP2020 located at the Rocky Mountain Biological Laboratory (RMBL). Heat desorption temperatures and GC-MS temperature ramping followed the same protocols outlined in Campbell et al. (2018). Resulting peaks from the GC-MS procedure were then integrated to estimate emission amount of each volatile chemical.

IV. Data Analysis

ANOVA was used to understand differences in each morphological or nectar trait in response to nitrogen treatments. Data were compared across the individuals who received nitrogen, control water, and ambient (unmanipulated environmental conditions) treatments. To understand floral trait response to soil moisture, linear regressions were plotted onto scatterplots of mean floral trait measurements for each individual plant and soil moisture.

Before analyzing our VOC data, we trimmed our GC-MS results via a set of filter parameters using the R package “bouquet” (Eisen et al. 2022) that selected for the most prevalent volatiles within each sample and eliminated volatiles found in air controls at amounts similar to those in samples. VOCs were selected if their peaks were present between 2 and 17 minutes of retention time, matched to a chemical in the NIST14 library (National Institute of Standards and Technology) with a similarity of at least 80%, were found in at least .02 samples (to account for indole) and contained a minimum peak area of 5×10^5 . We also compared VOCs to ambient air samples to eliminate contaminants. Only VOCs that were at least 3 times more prevalent in floral samples than ambient air samples and passed t-tests with a false discovery rate were used for analysis. Prior to analyzing our VOC results with statistical tests, we decided to square root each peak area, a measure of emission amount, to account for extremely large values. After corrections, to analyze the multivariate VOC results from our GC-MS procedure, we used a canonical analysis of principal coordinates (CAP) to visualize distinction in total VOC profiles. CAP analyses were used to visualize the differences amongst nitrogen treatment groups and across the observed soil moisture gradient in *I. aggregata* and hybrids. From our list of most prevalent volatiles, we then constructed an array of boxplots comparing emissions across

different treatments. We used these graphs to find VOC peak areas that appeared to be affected by treatments, which we tested with two-way ANOVA with the factors of treatment and day versus night. We also compared the effect of nitrogen between populations with two-way ANOVAs comparing treatment and site. We also used non-linear regressions to understand the relationship between VOC emission and soil moisture.

RESULTS

At the end of the study, a total of 247 flowers across both sites were measured for floral morphological traits. These flowers represented 56 *Ipomopsis aggregata* flowering individuals and 34 hybrid flowering individuals. For volatile collection, we measured 39 day samples and 38 night samples at the *I. aggregata* site and 11 day samples and 50 night samples at the hybrid site. Unfortunately, some individuals did not have fully opened flowers during the time of volatile sampling and therefore did not contribute to the total sample size. Of the 20 expected nitrogen samples (accounting for one sample each during the day and night), 6 samples were unable to be collected. For water control, only 4 samples were not collected.

The additions of dissolved ammonium nitrate fertilizer and water did not have a significant effect on the soil VWC at either site ($p > .05$), but the soil was generally wetter at the *I. aggregata* site (Figure 1). The mean soil moisture was 5.89% (SE = .13) at the lower *I. aggregata* site and 4.42% (SE = .20) at the hybrid population site. The nitrogen treatment significantly increased the chlorophyll index ($p < 0.0001$ for treatment effect in two-way ANOVA with the factors of treatment and site). Plants that received nitrogen averaged 0.41 for ChINDI compared to 0.32 and 0.30 for unmanipulated plants and plants that received water only (Figure 2).

Floral Morphological and Nectar Traits

Overall, most floral morphological traits were unaffected by the nitrogen treatments (Figure 3). Corolla length, style length, maximum and minimum anther position, and sepal width all exhibit minor differences across treatment groups that could not be explained by the treatments themselves ($p > 0.05$). This was true in both *I. aggregata* and hybrid populations. This same suite of traits was also unaffected by soil moisture (Figure 4). Linear regressions of trait measurements on VWC revealed weak relationships with all regression analyses having a p-value greater than 0.45. Regressions on VWC yielded an R^2 value less than 0.02 for traits of *I. aggregata* and hybrids. Therefore, floral morphological traits had no detectable correlation with soil moisture. Corolla width, however, differed significantly across treatments in hybrids. At the hybrid site, ambient plants had narrower flowers than those receiving extra water or nitrogen (ANOVA $p = 0.001$). Corolla width at the *I. aggregata* site also averaged higher in those treatments than for ambient plants, but the differences were not statistically significant ($p = 0.11$).

The results of floral nectar trait measurements primarily aligned with that of floral morphological traits (Figure 5). Nitrogen additions appeared to have no significant effect on nectar production in both *I. aggregata* and hybrids ($p > 0.14$). Nectar concentration stayed relatively consistent across treatment groups of *I. aggregata* ($p = 0.45$). No significant effects of treatments were found for the nectar traits at the hybrid site ($p > 0.42$). A noticeably wider variation in both nectar production and concentration was present in the water control group. The response of nectar traits to the observed soil moisture gradient was insignificant ($p > 0.1$), just as

it was for floral morphological traits (Figure 5). Nectar traits also exhibited linear regressions with R^2 values close to zero, suggesting no correlation in relation to soil moisture.

Floral VOC Bouquets

The floral volatile bouquets of *I. aggregata* contained a total of 63 VOCs that passed through our selective filters. From these 63 VOCs, we further selected 15 VOCs with a total peak area across all samples of at least 1×10^7 and manually included indole to produce a heatmap demonstrating relative emissions of each compound (Figure 7). Multivariate CAP analyses of *I. aggregata* and hybrid VOC profiles revealed no significant difference among treatment groups ($p > 0.45$), but day and night samples differed significantly ($p < 0.001$; Figure 8). This is mirrored by the CAP plot, where a clustering of points suggests little to no distinction across treatment groups is present. Additionally, ellipses representing the trends of each treatment group tended to overlap each other. Overall, floral scent profiles were separated predominately by day and night differences, which is aligned with the x-axis of the CAP analysis. However, when five prominent VOCs of *I. aggregata* were assessed individually, alpha-pinene emissions increased more at night in the nitrogen treatment ($p = 0.01548$ for the interaction of treatment with time of day; Figure 9). A similar result was obtained for beta-myrcene (interaction $p = 0.02188$, but no significant interactions were detected for beta-ocimene, alpha-farnesene, or caryophyllene ($p > 0.05$). When testing an interaction between treatments and population sites for night time emissions, alpha-pinene ($p = 0.036$) and beta-myrcene ($p = 0.01$) emissions were found to respond to nitrogen more in *I. aggregata* than in hybrids. At the hybrid site, none of these volatiles showed significant interactions between treatment and time of day (all $p > 0.05$). When investigating the indole results, we found that the filtering program only detected indole in two hybrid VOC samples, both taken at night.

After investigating the effect of soil moisture on *I. aggregata* VOC profiles, the CAP ordination plot again revealed only a distinction between day and night volatiles and not with soil moisture (Figure 10). Point distribution appeared random across the y-axis associated with the observed VWC distribution. When we took a closer look at each volatile with respect to soil moisture, it was clear that night collections demonstrated a wider variation in emissions than day time collections did (Figure 11). Alpha-pinene, alpha-farnesene, beta-myrcene, eicosane, and longifolene all exhibited a trend of relatively high peak area at low VWC as did the total peak area count. There is a similar, but less intense, effect on peak area at high VWC. However, these observations should all be taken with the caveat of wide confidence intervals.

DISCUSSION

Floral Morphological and Nectar Traits

Of the eight investigated floral morphological traits, only corolla width exhibited a response to our three nitrogen experiment treatments. In *I. aggregata* individuals, the resulting effect of nitrogen addition was not significantly different in corolla width when compared to ambient and water control groups, meanwhile hybrid individuals saw an increase in corolla width in both the water control and nitrogen groups. Corolla width has been shown to influence the ability of pollinators, especially hummingbirds, to extract nectar, judge the value of floral nectar rewards, and remove pollen from male-phase flowers (Melendez-Ackerman 1997; Campbell et al. 1997). Previous studies also investigated the effect of nutrient additions on *Ipomopsis aggregata* flowers (Burkle & Irwin 2009). They employed a more robust fertilizer containing

nitrogen, phosphorus, and potassium, which elicited a slightly greater response in corolla width of *I. aggregata* than our ammonium nitrate fertilizer. However, we did observe this change in corolla width within hybrids. These findings provide support for a plastic response in the corolla width of *Ipomopsis* under increased resource availability. However, it also appears that the addition of water could influence corolla width. Within the hybrid population, both the water control and nitrogen treatment groups produced wider flowers than the ambient group, which suggests that it was the 400 mL of water per week causing this response. Previous studies of the effect of water on corolla width have produced a variety of results, with Campbell and Wendlandt (2013) not finding an effect, but Powers et al. (2022) finding wider flowers with greater summer precipitation. In either case, due to this trait's relationship to hummingbird pollination, an increase in corolla width effect could serve to positively influence the abundance of hummingbird interactions with *I. aggregata* and hybrids, creating more opportunity for future cross pollination.

In contrast to morphological traits, all nectar traits of *Ipomopsis* were unaffected by more available resources. A lack of response in nectar quality and quantity may conflict with potential effects on corolla width, as the wider corollas induced by more nitrogen would not necessarily contain more nectar. Given that an increase in corolla width but not an increase in nectar trait are associated with increased nitrogen access, there may be potential for interactive effects on pollinator attraction, which would then warrant further study.

Surprisingly, not a single floral morphological or nectar trait showed a response to differing soil moisture conditions. These findings appear counter to previous documented trends that relate these same traits to changes in snowmelt date (Powers et al. 2022), which is often considered to dictate the water dynamics of soil throughout the growing season of mountain ecosystems. We, however, only made two measurements of soil moisture for each plant throughout the entirety of the summer. Additionally, the first measurement was collected in late June-early July, which was just around the beginning of the regional monsoon season while the second measurement came after weeks of near-daily rainstorms. This temporal limitation in our data may have limited our understanding of soil moisture dynamics, thus preventing us from noticing any relationship between soil VWC and floral trait measurements. A broader sense of soil moisture may have been especially valuable for floral traits in comparison to VOCs because of the cumulative investment required for these traits.

Floral VOCs

This study marks the first time floral VOCs were investigated in *Ipomopsis* under differing nitrogen conditions. When considering floral bouquets as a whole, treatments did not demonstrate any significant effect. In both of our CAP analyses, this was supported by the observed homogeneous distribution of samples. Not only was floral scent composition consistent across treatments, but so were emissions by the majority of individual floral VOCs. Total emissions stayed relatively consistent in both hybrids and *I. aggregata*. Alpha-pinene and beta-myrcene appeared to show larger night time increases with nitrogen additions in *I. aggregata*, but this was still not enough to demonstrate any difference in total emissions or in total bouquet composition. A study of nutrient additions to another alpine-subalpine species, *Arabis alpina*, found that total emissions were increased when exposed to more resources (Luizzi et al. 2021). While this result was not shared in our study, both findings agreed on a lack of response in floral composition, suggesting that individual VOCs, except for two monoterpenes in our study (alpha-

pinene and beta-myrcene), may uniformly respond to differing nutrient scenarios. Responses of these monoterpenes were more evident in the *I. aggregata* than the hybrid population, possibly because those plants are less water-limited (Wu and Campbell 2007). As predicted by our initial hypotheses, *I. aggregata* did exhibit a unique response to nitrogen treatments when compared to the hybrid population. Further study into the response of the other parent species, *I. tenuituba*, would be necessary in order to determine if this is a transgressive or intermediate response with respect to parent species.

Unfortunately, our filtering parameters for VOCs meant that we were unable to identify many peaks of indole within hybrid samples. This resulted in us only seeing two cases of indole emissions within our data. Further analysis of the data should employ manual identification by searching for the top ion of indole to gain an accurate understanding of indole emissions in the hybrid population.

Our study was not able to identify any significant relationship between floral VOCs and soil moisture. This is surprising, considering that drought conditions were previously shown to alter emissions with a well-supported non-linear relationship (Campbell et al. 2019). While our data does suggest a non-linear trend at night with increases in emission at low and high soil moisture, this relationship seems to be poorly fit judging by the confidence interval. This suggests that a snapshot of soil moisture may not be enough to understand its influence on VOCs. In the previously mentioned study, plants were tracked throughout their dry-down process, meaning that soil moisture history was also considered in the study.

Conclusion

This study aimed to understand the potential for nitrogen and soil moisture to affect floral phenotypes in *Ipomopsis aggregata* and hybrids. Evidence suggests that nitrogen produces significant changes in morphology of flowers but lacks a similar level of influence over VOCs. Soil moisture appeared to lack much effect at all, which may warrant a different approach to understanding VOC response to water availability. In either case, this work demonstrates the importance of abiotic factors in determining the future of flowers. By knowing more about how our changing environment will affect floral traits, we can better anticipate changes in the relationships that depend on these flowers. A change in flowers will likely translate to a change in pollinator interactions, which poses consequences for these delicate pollinator systems.

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FIGURES

Figure 1. The effect of treatments on volumetric water content (VWC) of soil (%). Individual plant measurements are indicated by each point. A greater number of soil moisture measurements were made at the lower *I. aggregata* site due to rocky soil conditions at the hybrid site. No significant difference is present between treatments.

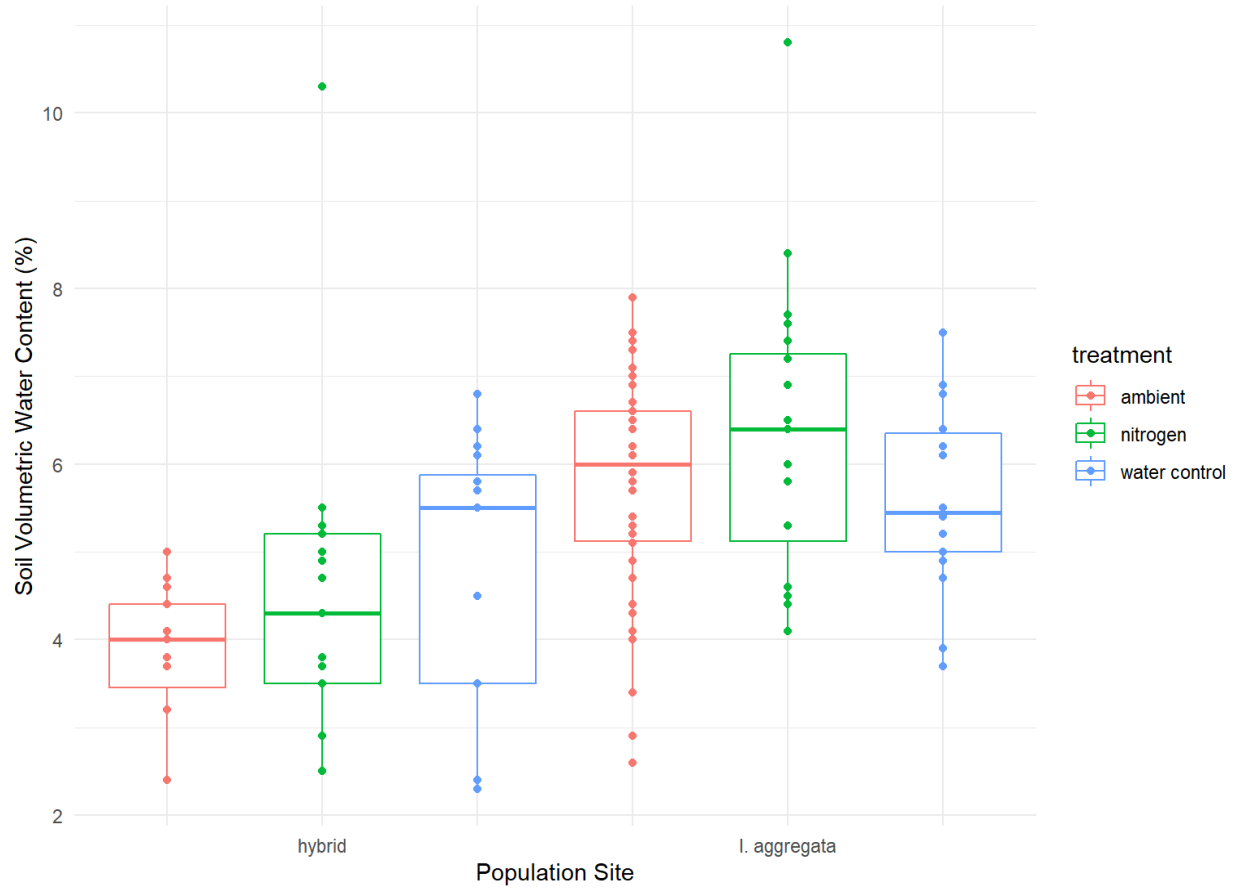


Figure 2. The effect of treatments on chlorophyll a content of leaf tissue. Individual leaves underwent reflectance spectrometry to gauge the accumulation of nitrogen by plants within the nitrogen addition group. Leaf nitrogen content is proportional to the chlorophyll a index present on the graph. A significant difference was observed across treatment groups ($p < 0.0001$).

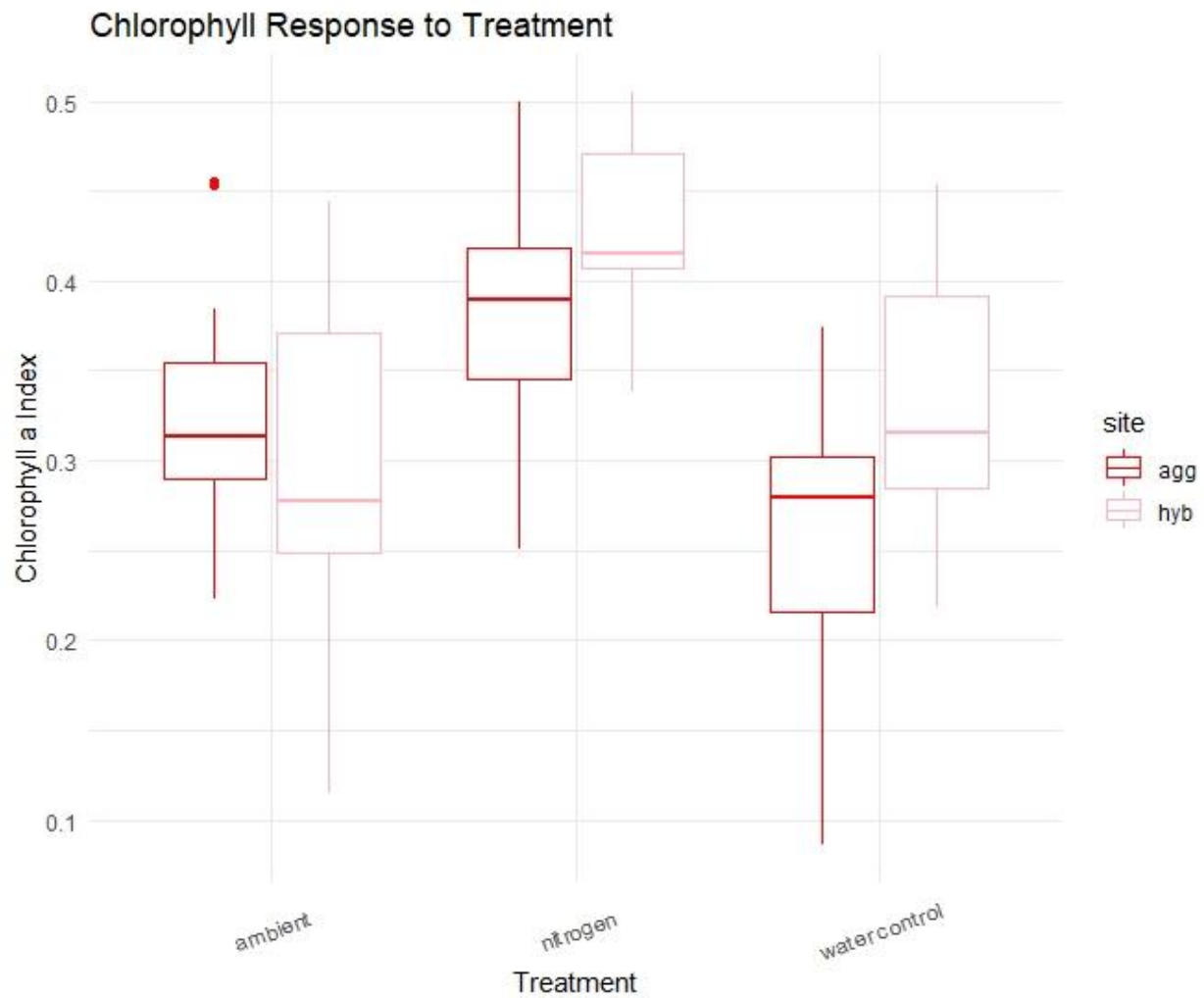


Figure 3. The effect of treatments on morphological floral traits of *I. aggregata* and hybrids. Each trait plays a significant role in determining plant fitness as discussed in Campbell et al. (1991) and Campbell et al. (2022). Individual plant measurements are indicated by each point. Corolla width differed significantly among treatment groups in hybrids. No other significant differences were detected.

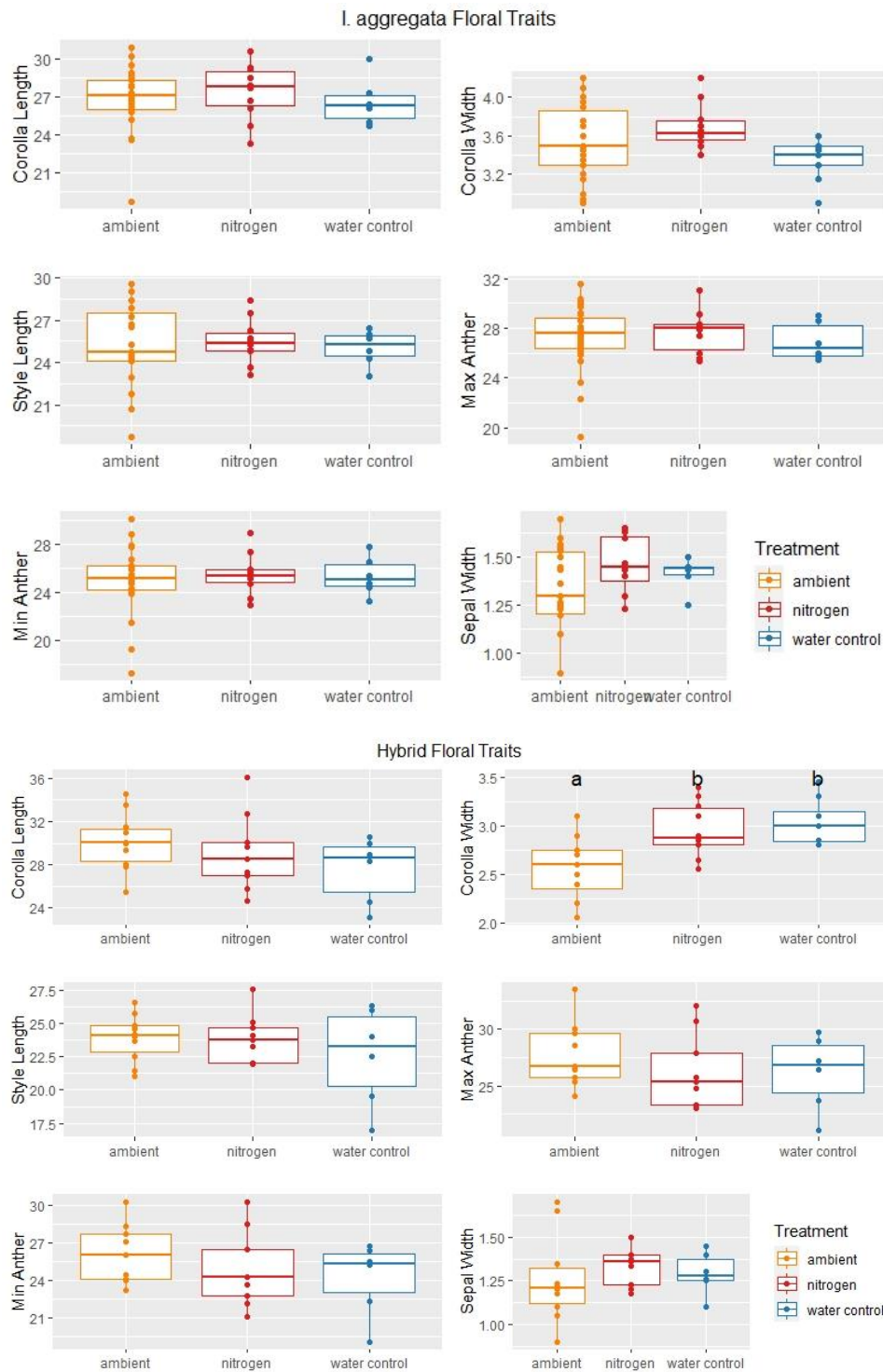


Figure 4. The effect of a natural observed soil moisture gradient on floral traits of *I. aggregata* and hybrids. Each trait plays a significant role in determining plant fitness as discussed in Campbell et al. (1991) and Campbell et al. (2022). Individual plant measurements are indicated by each point. Linear regressions were made to best fit each scatterplot. They are accompanied by a grey area indicating the confidence interval of the regression. No significant correlation was found between floral traits and soil moisture.

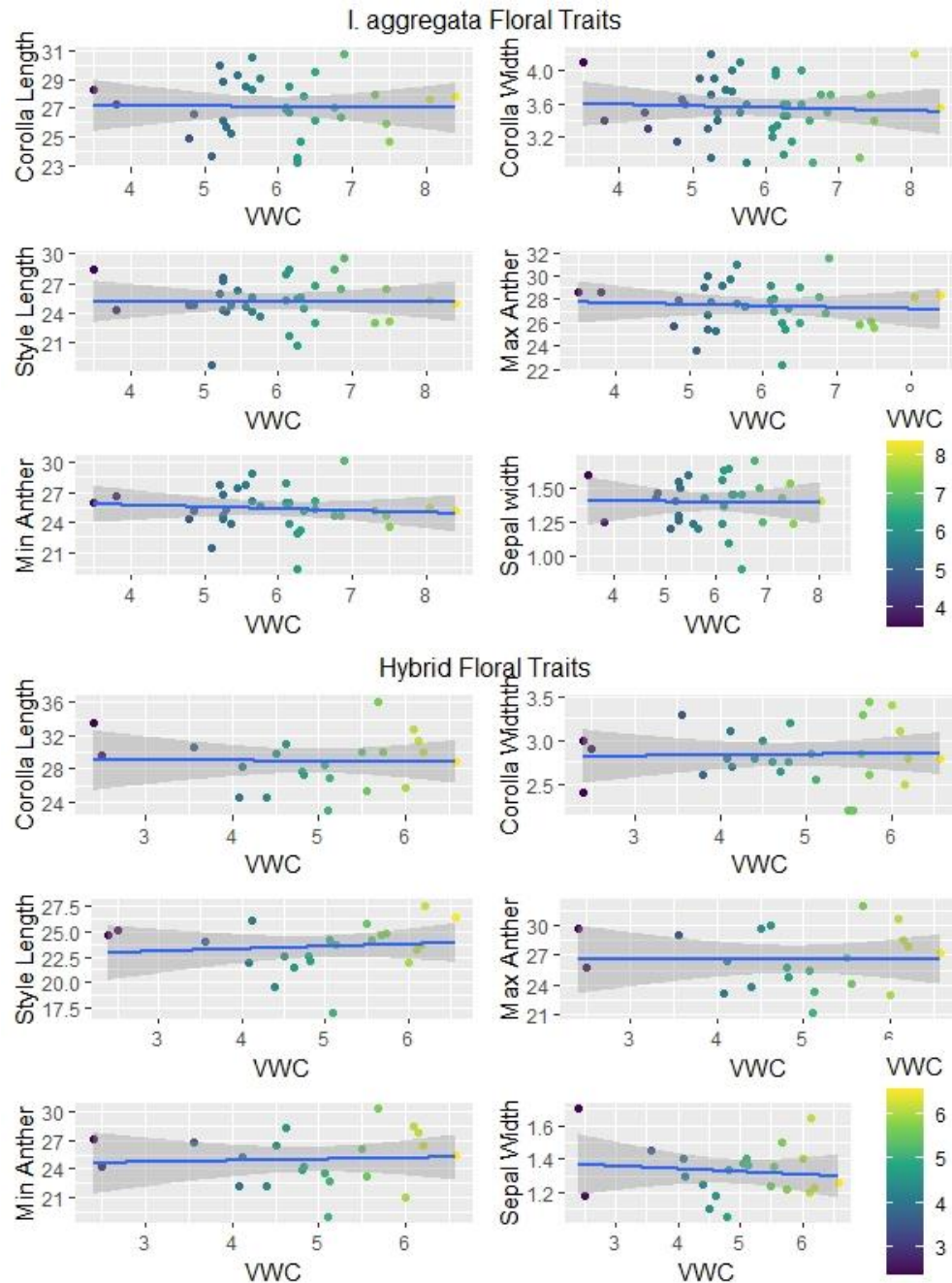


Figure 5. The effect of treatments on nectar traits of *I. aggregata* and hybrids. Individual plant measurements are indicated by each point. Nitrogen had a significant and positive effect on nectar production in *I. aggregata*. No other significant differences were found between the three treatment groups.

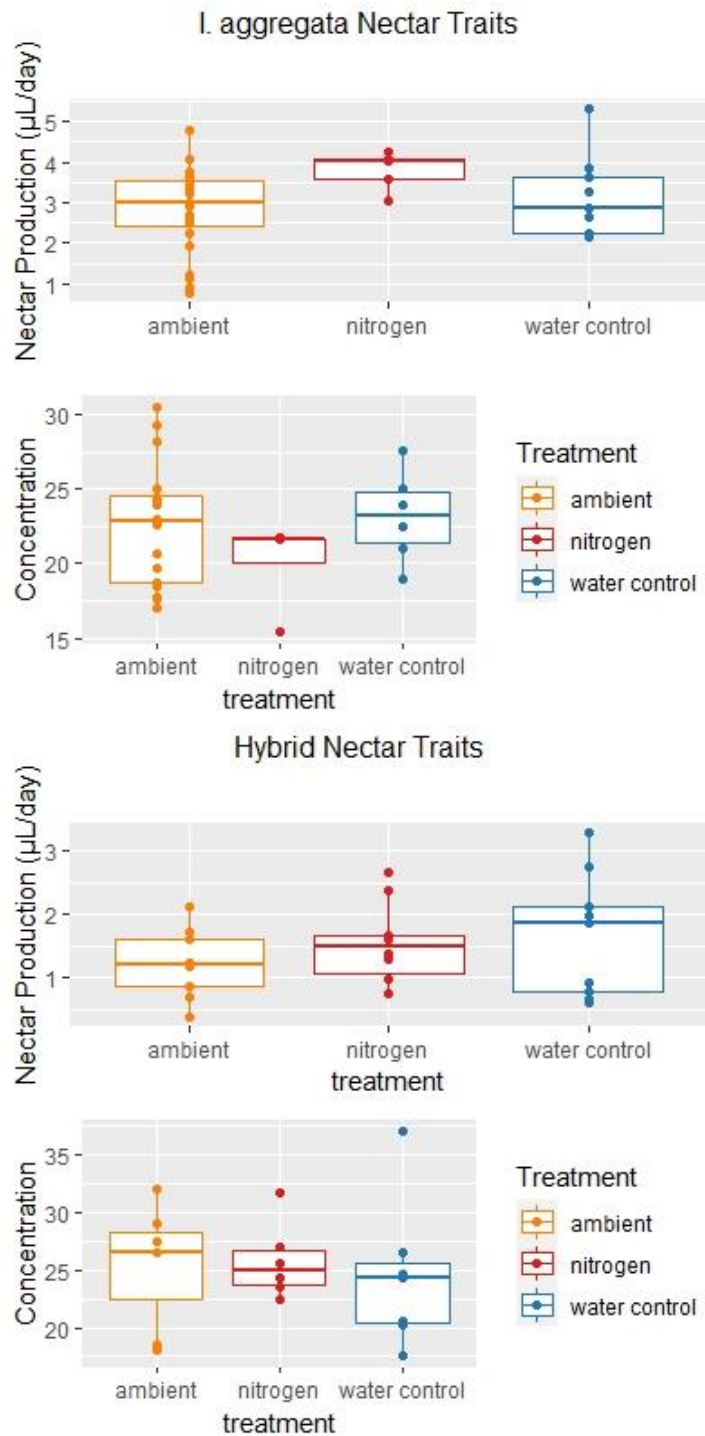


Figure 6. The effect of a natural observed soil moisture gradient on nectar traits of *I. aggregata* and hybrids. Individual plant measurements are indicated by each point. Linear regressions were made to best fit each scatterplot. They are accompanied by a grey area indicating the confidence interval of the regression. No significant correlation was found between floral traits and soil moisture.

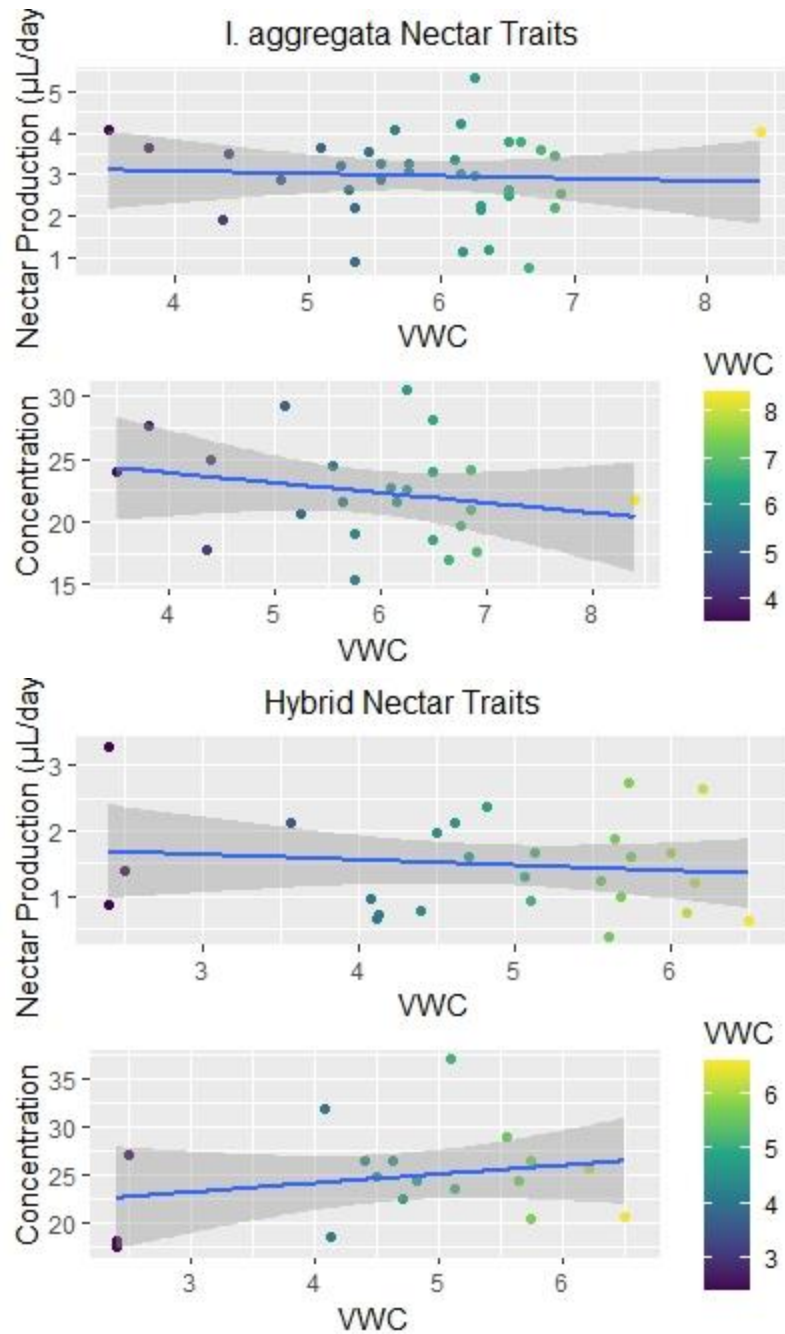


Figure 7. A heatmap showcasing the distribution of volatile emissions with respect to population site, treatment, time of collection, and soil VWC. On the right of the heat map is a list of all 16 VOCs that passed our set of filters. On the left is a descriptive tree displaying the relatedness of volatiles with respect to their emission amounts. Lighter colors within the heatmap indicate a high level of emission within that respective sample.

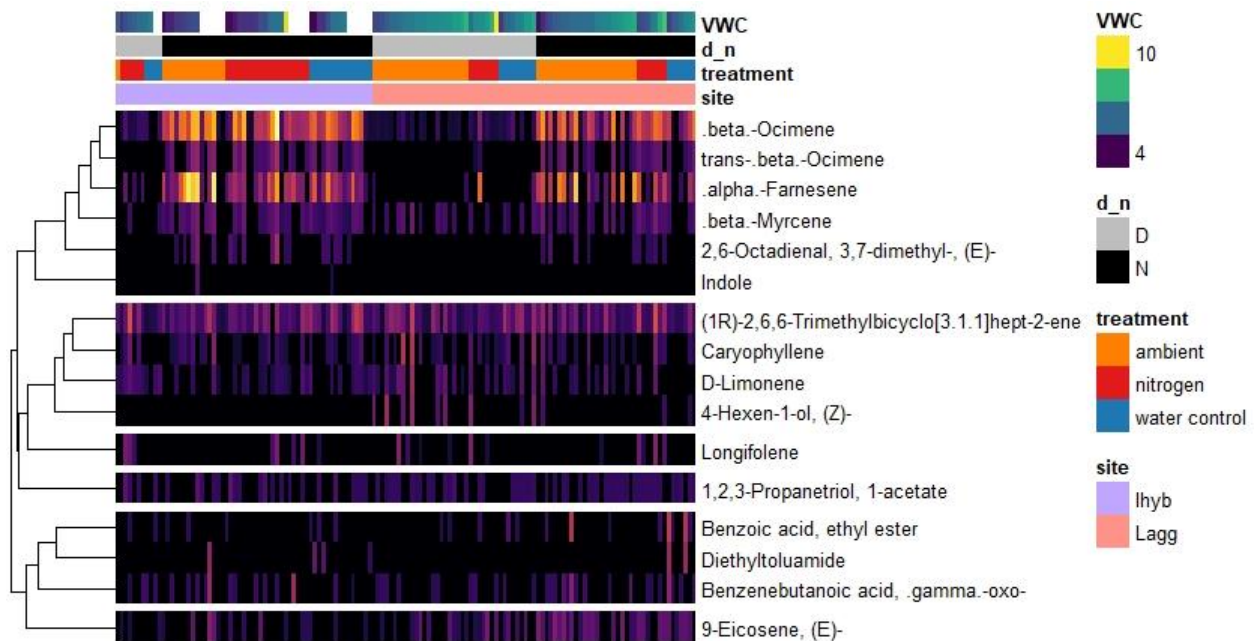


Figure 8. CAP plots investigating the interaction of treatments and temporality in VOCs of *I. aggregata* and hybrids. Points are colored by both treatment and time and are shaped by time. Ellipses highlight the grouping trends among nitrogen, water control, and ambient treatment groups. Finally, arrows indicate the direction that specific predictors and their related points are organized.

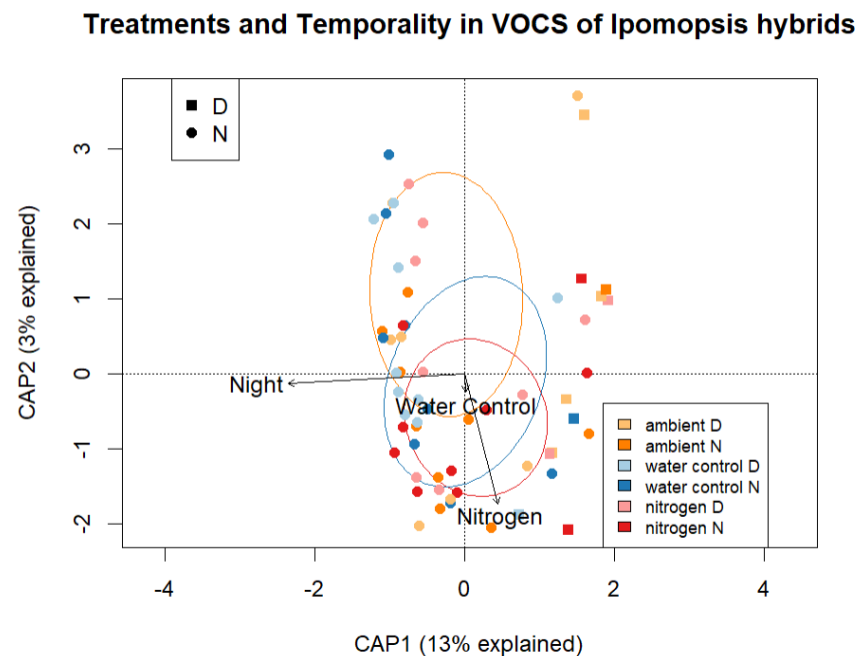
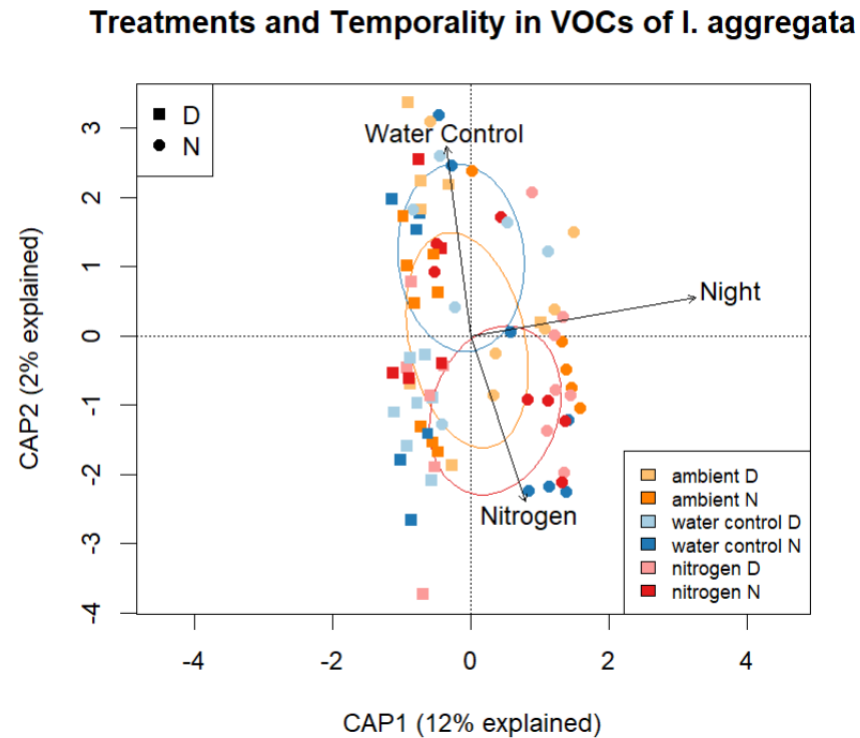


Figure 9. The effects of treatments and time on alpha-pinene and beta-myrcene peak areas of *I. aggregata* and hybrids. Alpha-pinene comprises the bulk of *Ipomopsis* floral bouquets. In *I. aggregata*, both alpha-pinene and beta myrcene showed significant interactions between treatment and time. No other significant differences were found between the three treatment groups.

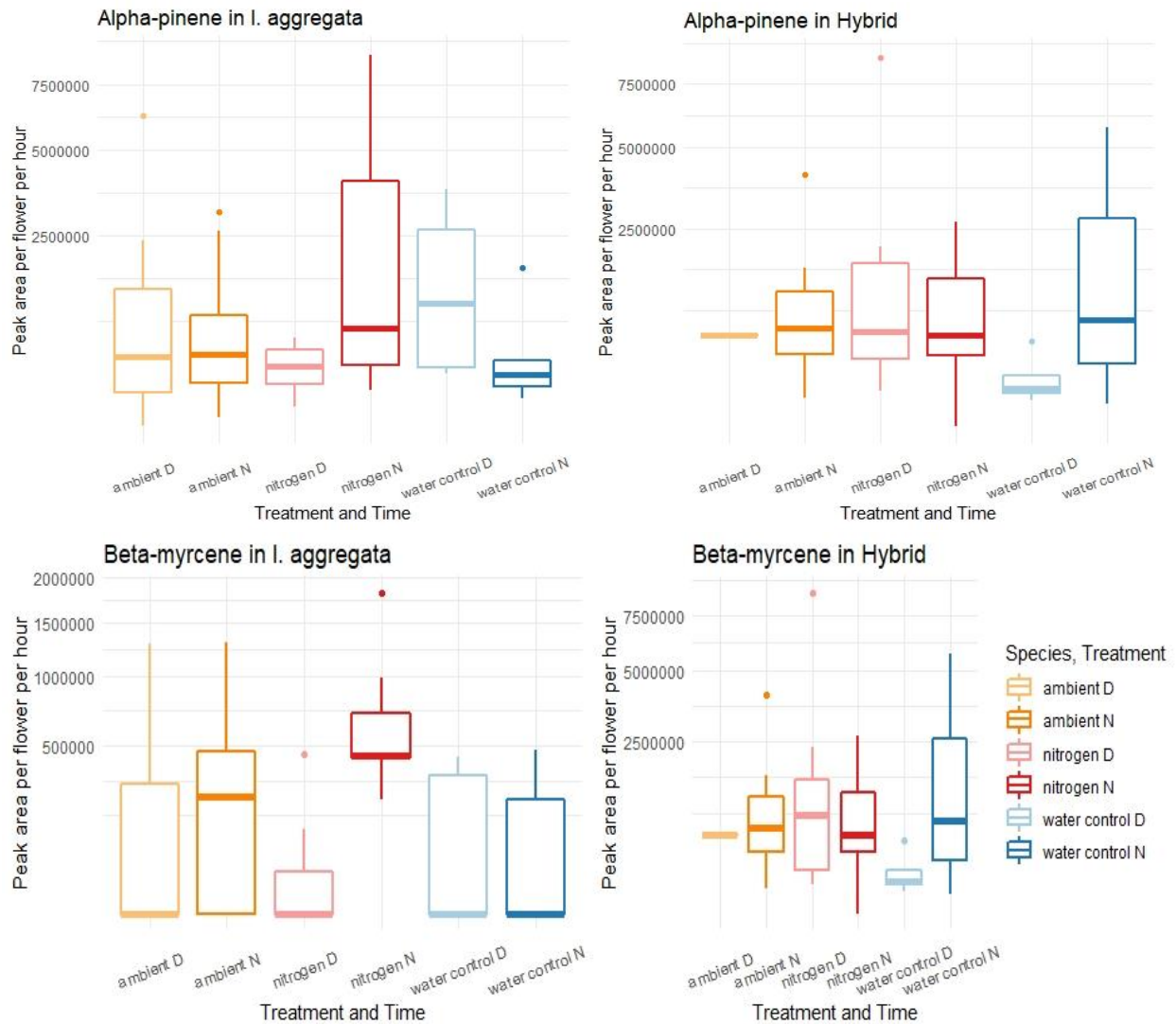


Figure 10. CAP plot investigating the interaction of soil VWC and temporality in VOCs of *I. aggregata*. Points are colored by VWC and are shaped by time. Lines are drawn between samples of the same plant to illustrate changes between day and night volatiles. Darker colors represent a lower VOC measurement and lighter colors represent a higher VOC measurement. Prevalent chemicals are included to indicate what direction relates to their increase, but due to lack of distinguishing groups, they have clumped in the middle. Finally, arrows indicate the direction that specific predictors and their related points are organized.

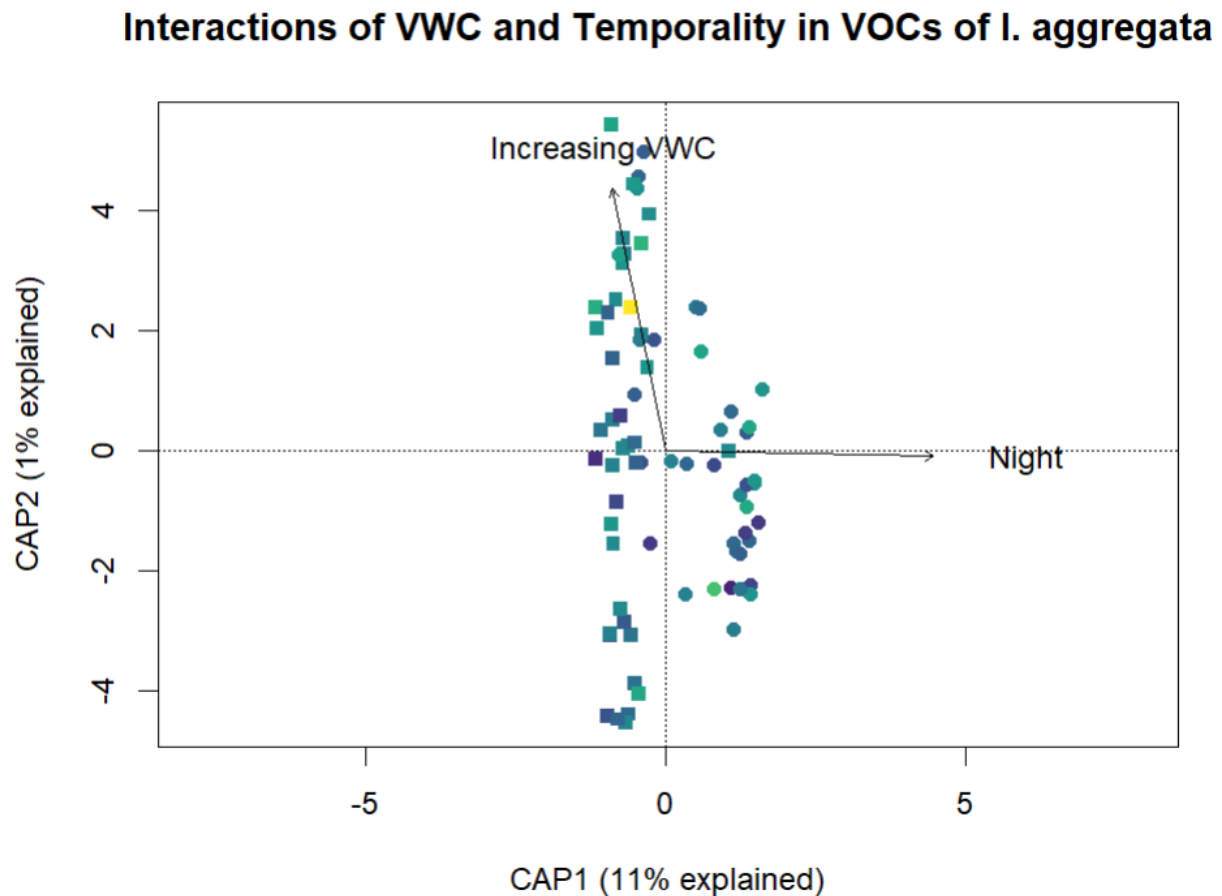


Figure 11. The effect of soil VWC on specific peak areas of *I. aggregata* VOCs. The first boxplot describes trends for the total peak area of each VOC sample. Each consecutive boxplot describes only the peak areas for that specific VOC. All plots are fitted with a non-linear regression surrounded by a grey area signifying the corresponding confidence interval. Data was colored by day and night collection time. No significant correlations were discovered between the three treatment groups.

