

# Nitrogen Composition in Native and Invasive Plants in Relation to Ant Mounds in Serpentine Grasslands

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A Thesis

Presented to the Environmental Studies Program of the University of Oregon

In partial fulfillment of the requirements

For the Degree of

Bachelor of Science

University of Oregon

June, 2019

An Abstract of the Thesis  
Elizabeth Baach for the degree of Bachelor of Science  
In the Environmental Studies Program to be taken June, 2019  
Title: Nitrogen Composition in Native and Invasive Plants in Relation to Ant  
Mounds in Serpentine Grasslands

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**Abstract:**

Serpentine grasslands are unique ecosystems that, due to their soil composition, prove to be areas where plant growth is difficult for most. With this particular soil composition having low levels of essential nutrients, and high levels of toxic minerals, plant endemism is particularly high in these ecosystems. However, there are processes in the ecosystem that make limiting nutrients, especially Nitrogen, more available, caused by ground dwelling organisms including ants. The particular organism of focus for this study is the harvester ant, *Messor andrei*, which creates mounds. These ant mounds are sites of nutrient upwelling, where nitrogen is made more available to plants growing on mound. Considering the ecosystem characteristics and processes occurring within, this project aimed to understand if there was a difference in nitrogen content in native and non-native plant species based on their on off mound condition. From this, two hypotheses were created that state there will be a significant difference between the different plant species based on their native/non-native status and functional group, and that there will be a significant difference between the on and off ant mound conditions. To test these, plant tissue samples were collected from seven species of different status and functional role from both on and off mounds at three different blocks in the Jasper Ridge Biological Preserve. The carbon to nitrogen ratios (C:N) were then calculated for these samples. Comparing the C:N of the different plant tissues there was found to be significant difference in C:N overall between the on and off ant mound condition. In addition there was found to be a significant difference between plant species with the lowest C:N being found in the native nitrogen-fixer and the non dominant forbs and a significantly higher C:N in the dominant forb and the non-native grasses.

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## **Introduction**

### Soil/Plant Relationship:

There is a complex and important relationship between plants and the soil they are in, much more than what meets the eye. Soil not only provides support and a medium for plants to grow, but it also has a primary role in how plants are able to attain water and nutrients. Soil has global variety and is impacted by a variety of factors, the most prevalent factors being climate, topography, parent material, time, and the organisms in and around it. These factors determine the texture class of the soil, ranging from clay to sand, smallest to largest respectively. The texture class of the soil has a great impact on the cation exchange capacity, especially when comparing surface area, as the greater surface area a soil has, the higher the cation exchange capacity will be. This process is extremely important to plant nutrient uptake as well as soil health. Using these concepts, and applying them to serpentine soils, it is easier to understand why these ecosystems are so unique and important.

### Serpentine Grasslands:



**Figure 1:** A photo of the serpentine grassland in Jasper Ridge Biological Preserve.

Serpentine grasslands are found globally, as it is defined as a place where grasses and forbs dominate a landscape with serpentine soil made from ultramafic rocks. This study, however, looks more specifically at California serpentine grasslands, occupying about 1% of California's landscape, are a unique landscape occupied by 10% of California's endemic species (Huenneke et al, 1990). Comprised of mostly grasses and forbs this ecosystem shows the presence of highly specialized species, as the conditions do not allow others to establish, until now. The soils in serpentine grasslands do not contain large amounts of essential nutrients for plant growth. The two most notable limiting resources being phosphorus and nitrogen. The other factor that makes establishment of plants difficult is the dangerously high levels of toxic minerals including iron, nickel and cobalt, among others. In ecosystems of this nature, changes in nutrient availability can be particularly devastating, allowing for the introduction, establishment, and domination of non-native invasives (Weiss, 1999).

One factor that is particularly important for the development of serpentine soils is the parent material this being ultramafic rock, more specifically serpentinite. This mineral subgroup of ultramafic rock is particularly well known for the high content of magnesium and iron, which

mixes into soil as the parent material is eroded. These elements, in large amounts are toxic to plants, and soils that contain large amounts are often uninhabitable by many species. This in combination with the low availability of essential limiting nutrient is what makes serpentine soils, and in result serpentine grasslands to have such high levels of endemism.

### Nitrogen in Serpentine Systems:

Nitrogen provides a vital role in any ecosystem. It is the major component of proteins and enzymes that allow cells of any organism to function. Nitrogen comprises the majority of the atmosphere, so why is it a limiting resource? In the atmosphere nitrogen is diatomic and unusable by plants. Nitrogen must first be fixed from its atmospheric form into ammonium and nitrates, the forms that plants are able to use. These processes are only able to be completed by certain form of bacteria that associate symbiotically with certain plants, which is the step that makes nitrogen a scarce resource and therefore limiting . In serpentine grasslands, the presence of nitrogen is even more limiting than in other ecosystems, which is why only certain plants have been able to grow there. The low levels of available nitrogen for plant uptake makes native plants that are resource conservatives thrive and other non-natives that are resource acquisitive not have the necessary nutrients to survive. While this would be the case in a static ecosystem, serpentine grasslands have processes that alter nutrient cycles, one of the most important for this study is nutrient upwelling by ground dwelling organisms, such as ants.

### Nutrient Upwelling by Ants:



**Figure 2:** Image of a harvester ant (*Messor andrei*) mound with plants growing on and around it (**left**). Eliza Hernández, Dr. Richard Hobbs and me pointing at another ant mound in Jasper Ridge Biological Preserve (**right**).

Resources in the form of nutrients is cycled through the soil layers by a variety of organisms and processes. One group of organisms that plays an important role in the cycling of serpentine grasslands is that of the ants. Just within the Jasper Ridge serpentine grassland there are 13 different species of ants, with many of these playing an important role in soil movement and plant community composition heterogeneity. The majority of ant species in Jasper Ridge are native, with only two being considered invasive. These invasive ants, however, have shown to

have no significant ecological impact on this grassland system. The species of focus for this work is the harvester ant (*Messor andrei*). Harvester ants have large black bodies with squared heads and have been observed to be a group forager, meaning they go out in groups to collect seeds and bring them back to the mound where they consume them (Jasper Ridge, 2019). These ants, through the creation of mounds, bring up deeper soil that was previously unavailable to plants and place it on the surface of their mounds. They also produce waste and have leftovers from the seeds they bring back and eat, which is another source of nutrients made more available on ant mounds. This allows the plants growing on the ant mounds to have increased access to nitrogen compounds, which could change ecosystem dynamics and potentially allow previously restricted non-native plants to invade and establish.

In addition to changing the nutrient availability within serpentine ecosystems, forager ants have also been changing plant abundance and distribution. When out foraging in groups the ants collect and then bring back seeds to their mound, where some are eaten and others are able to establish and grow. The ants do not collect seeds based on species density and therefore have a significant impact on plant abundance and distribution within the serpentine grassland (Hobbs, 1985). The movement of plants and nutrients through serpentine grasslands has changed the plant community composition and dynamics as well as has allowed establishment on non-native generalist grasses.

### Establishment and Takeover of Invasive Plants:

Changing ecosystems, and especially changing availability of nutrients, allow generalist invasive plants to establish in serpentine grasslands and potentially outcompete the native, endemic plants. Limiting resources, when added in abundance, while seemingly beneficial due to the increase in ability of plant growth, is in reality detrimental as it allows a few specialized, usually non-natives, to grow exponentially and outcompete the other, native species and ultimately cause great change to the ecosystem of focus. Serpentine grasslands, because of the already low levels of limiting resources, especially experience the effects of the addition of nutrients to the same levels as is seen in freshwater eutrophication (Huennke et al., 1990).

### Project Goal:

Determine if there is a significant difference in nitrogen content in native and non-native plant species by analyzing samples from both on and off ant mounds in the serpentine grasslands.

### Questions and Hypotheses:

How will different plant species in serpentine grasslands differ in resource acquisition?

$H_{01}$ : There will be no significant difference in carbon nitrogen ratios between species.

$H_{A1}$ : There will be significant differences between the different plant species based on their native/non-native status and role in the ecosystem.

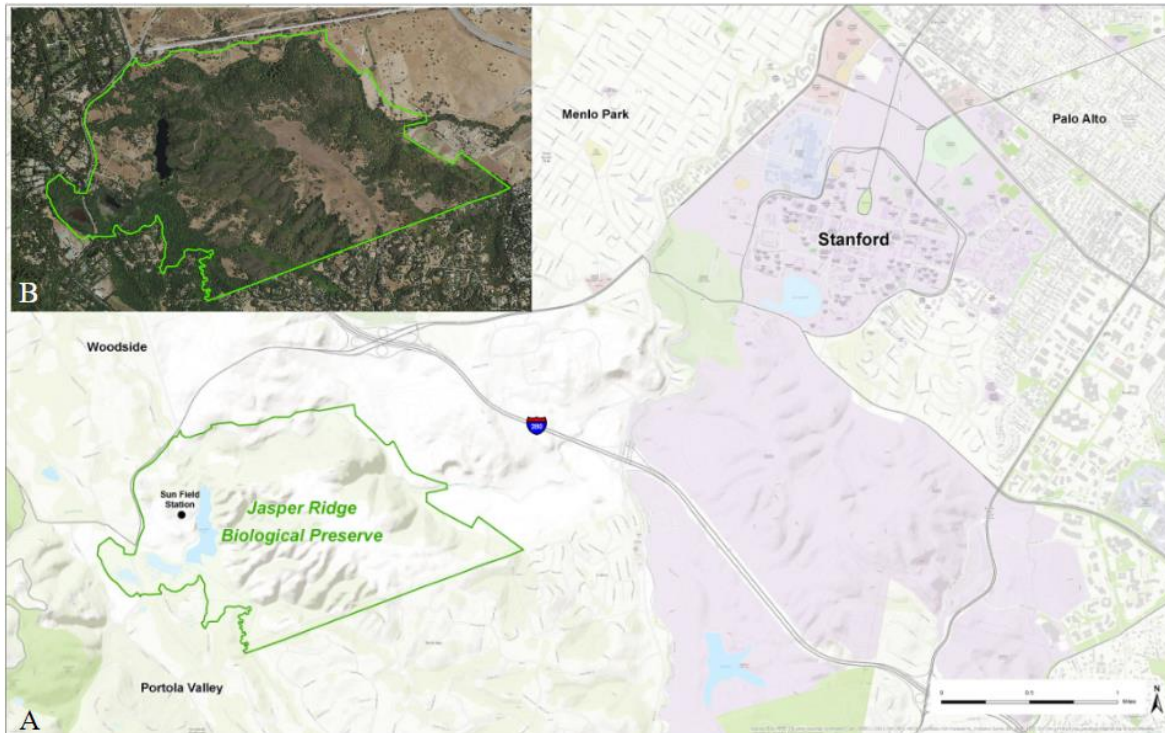
How will plant species respond to on and off mound conditions?

$H_{02}$ : There will be no significant difference in carbon nitrogen ratios between the on/off mound conditions.

$H_{A2}$ : There will be a significant difference between the on/off ant mound treatment with the on mound condition having a lower carbon nitrogen ratio.

## Methods

### Study Area:



**Figure 3:** A. Map of the location of Jasper Ridge Biological Reserve in Relation to Palo Alto, California and the Stanford University Campus. B. A satellite view of Jasper Ridge Biological Reserve showing the natural landscape and topography (Jasper Ridge, 2019).

The serpentine grassland from which the plant samples were collected was from Jasper Ridge Biological Reserve outside of Palo Alto, California near the campus of Stanford University. This reserve is in the lower foothills of the Santa Cruz mountains in the base of the San Francisco peninsula. Jasper Ridge spans around 483 hectares (1193 acres) and lies on a topography varying from altitudes of 61.6 to 211.5 meters (202.1 to 693.9 feet). The climate of the area has been defined as Mediterranean, which consists of hot dry summer and cool wet winters, and has a mean annual precipitation of 65.2 centimeters (26 inches) and a mean growing season temperature of 56 F. This climate, in addition to the Los Gatos loam soil type, are the factors that have the serpentine grasslands at the Jasper Ridge Biological Reserve so remarkable.

## Focal Plant Species:

### Nonnative grasses:



*Bromus hordeaceus*

Soft chess is a grass species that, while native to the Mediterranean basin, has naturalized every continent except Antarctica. Interestingly, this grass species grows best in areas that are less fertile and has adaptations to both varying temperatures and water availabilities (INaturalist.org, 2018).



*Lolium multiflorum (Festuca perennis)*

Italian ryegrass is native to temperate Europe, although it has spread and naturalized much of the Northern Hemisphere. Because this grass grows so readily in temperate climates it has been considered a weed in agricultural settings and an invasive in natural ones (INaturalist.org, 2018).

### Native Nitrogen fixer:



*Lotus wrangelianus (Acmispon wrangelianus)*

Despite what its common name may suggest, the Chile Trefoil is not from Chile but rather is native to California, Oregon and Nevada. This annual legume forms a symbiotic relationship with *Rhizobium* a nitrogen-fixing bacteria that forms root nodules with plants in the legume order, *Fabaceae* (INaturalist.org, 2018).

### Native forbs:



*Plantago erecta*

Dot-seed plantain, also known as California plantain, is a small forb native to Oregon and California where it grows in sandy, clay, and serpentine soils. This species is a host for the Edith's checkerspot butterfly, which is currently listed under the Endangered Species Act (INaturalist.org, 2018).



*Layia platyglossa*

Tidytips is a native of low-elevation dry environments of California but has been spread more due to its use as an ornamental plant in gardens and as a source of pollinator habitat in restoration projects (INaturalist.org, 2018).





*Lasthenia californica*

California goldfields is an annual flowering forb of the daisy family native to California and the surrounding areas. Large populations of this species blooms all at once, which is how this species gained its name as it makes the fields into a golden hillside (INaturalist.org, 2018).

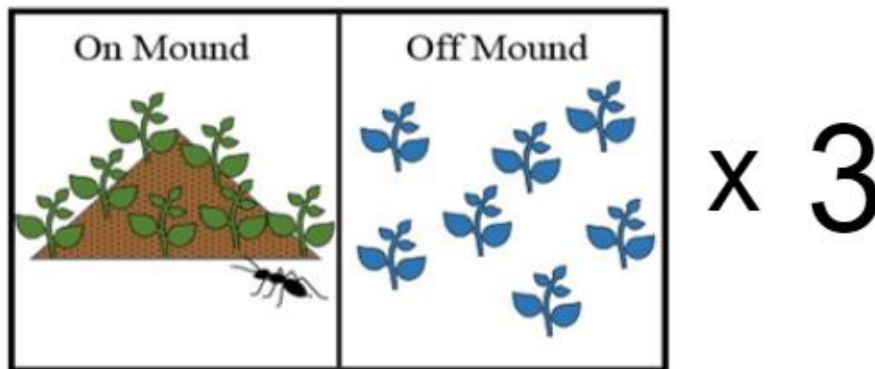


*Hemizonia congesta* spp. *Lutescens*

Yellow hayfield tarplant is endemic to the San Francisco area of California where it grows up to 4600 feet in elevation in grassy areas. It is a host for two moth species, *Heliothis phloxiphaga* and *Cyclophora dataria* (California Native Plant Society).

Sampling Design:

42 leaf tissue samples were collected on April 9, 2018 from Jasper Ridge Biological Preserve. This collection consisted of six samples of each of the seven species from both on and off ant mounds. Slope, mound size and aspect were all similar between blocks, to control for potential influence.



**Figure 4:** shows the experimental design of collection, with three blocks each having the seven species collected from both on and off ant mounds at the site.

Leaf Tissue Processing and Analysis:

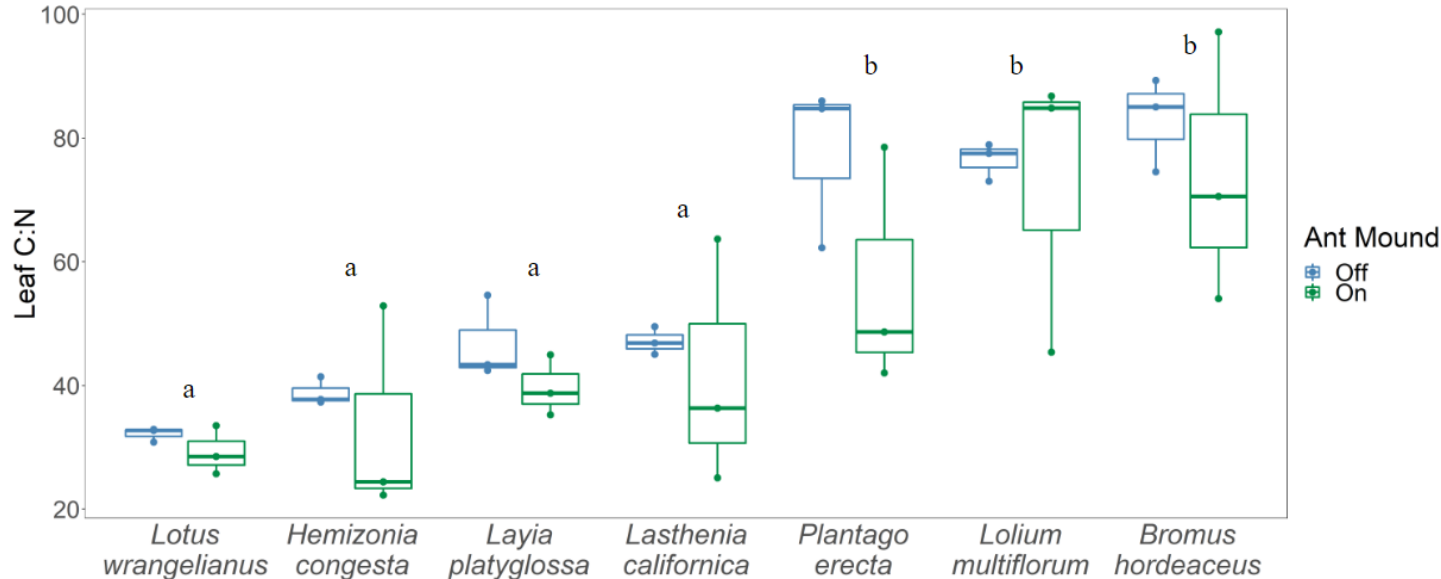
After samples were brought back from the field, I dried them at 60 Celsius for 3 days. I ground up leaf tissue using a ball mill until the material was a fine powder. Using a microbalance, I weighed 2-4 milligrams of ground tissue per sample and encapsulated samples with tin. Samples were analyzed for percent weight of carbon and nitrogen using an Elemental Analyzer. Carbon to nitrogen ratios were calculated by dividing percent carbon by percent nitrogen.

Statistical Analysis:

To test whether C:N differed between species and on and off ant mounds, linear mixed-effects model was used. To run a linear mixed-effects model to analyze C:N, we used the lme

function of the nlme package (Pinheiro et al., 2019). The linear mixed-effects used the ant treatments and species as fixed effects, and block as random effect. Least square means were calculated for pairwise comparisons between species using the package lsmeans (Lenth, 2016). This was a pilot study, and a caveat of this was that statistical analyses here are preliminary in nature due to low sample size and the linear mixed effects model not meeting assumptions in normality and homogeneity of variance, refer to Supplement (#). We did not include a species ant mound interaction in the model due to too few replicates.

## Results



**Figure 5:** Carbon to nitrogen ratios (C:N) of leaf tissue between species on and off ant mounds. Significance between species is distinguished using ‘a’ and ‘b’ and using p-values noted in Table 2. There was a significant difference in C:N on and off ant mounds (Table 1; p-value: 0.029).

There was found to be a significant difference between the on/ off mound condition for all species ( $p < .0001$ ). This means that plants respond to variation in the environment. If more nitrogen is made available by ant mounds, plants located on these mounds will have more nitrogen in their tissue and therefore lower carbon nitrogen ratios.

The highest carbon nitrogen ratios were found in the two non-native grasses, *Bromus hordeaceus* and *Lolium multiflorum*, and the dominant forb, *Plantago erecta*. These showed to have no significant differences between each other, however, they did show a significant difference in carbon nitrogen ratios from the other samples plants. The lowest carbon nitrogen ratio was found in *Lotus wrangelianus*, the native nitrogen-fixer. This is likely due to its symbiotic relationship with the nitrogen-fixing *Rhizobium* bacteria and the subsequent increased access to nitrogen. There was no significant difference in carbon nitrogen ratios between *Lotus wrangelianus* and the three less dominant native forbs, *Layia platyglossa*, *Lasthenia californica*

and *Hemizonia congesta*. There was a significant difference between this group and the others, as mentioned previously.

There was also found to be a difference in heterogeneity on and off ant mound, with on mound having greater amounts of C:N variability.

On, off mound treatment was determined to be significant for all species, however due to a limited sample size, significance was not able to be determined within each species.(p-value: 0.029)

## Discussion

These results have a variety of implications for these systems and can provide helpful information for extensions of this study and future directions.

The significant difference between on and off mound condition means that plants respond to variation in the environment. If more nitrogen is made available by ant mounds, plants located on these mounds will have more nitrogen in their tissue and therefore lower carbon nitrogen ratios.

The significant difference between plant species groups in C:N shows that there is a significance to a plant's native/ non-native status as well as to its functional group. This also has the potential implication of a lack of change with the increased presence of the non-native grasses, in terms of nutrient dynamics, as the dominant forb and the non-native grasses showed to not have significantly different C:N.

There was found to be greater variation of C:N on the ant mounds in comparison to off ant mounds. One possible explanation could be the age of the ant mounds, with the older mounds having greater accumulation of nutrients due to the increased duration of ant disturbance. Another could be variation in ant mound plant composition, unintentionally put in place by ant seed foraging (Hobbs, 1985).

Another factor potentially influencing the serpentine grassland landscape is the differing rates of decomposition occurring with varying carbon nitrogen ratios. The lower the carbon nitrogen ratio, the higher the decomposition rates will be. This is more likely as fungi, bacteria and other decomposers prefer systems with more nitrogen and will decompose these areas at higher rates in comparison to those with less available nitrogen. Ants are also moving large amounts of soil and organic matter into their mound which removes available nutrients from the surrounding, thus slowing decomposition rates (Schowalter, 2016). Having such a large contrast between on and off ant mound nitrogen availability could have large impacts on the grassland ecosystem.

Increasing development and fossil fuel use, increases rates of nitrogen deposition in addition to the other detrimental global climate trends. While this process adds more nitrogen to the soil and therefore the ecosystem in total, nitrogen deposition is much different than nitrogen fixation. The molecules created from nitrogen deposition include oxidized nitrogen, which while including nitrates, also includes other toxic molecules and acids. One of the major effect of nitrogen deposition is the acidification of soil which reduces the availability of other necessary

nutrients. The soil acidification reduces the cation exchange capacity of the soil, which reduces the ability of soil to hold onto certain nutrients and thus allows for greater potential of leaching and loss (Air Pollution Information System, 2016).

## Acknowledgements

I would like to thank Dr. Lauren Hallett and Eliza Hernández for helping me every step of the way. The assistance and encouragement I received from you both made this project so amazing and provided me with so many opportunities.

I would also like to thank the rest of the Hallett lab for your insight and motivational words to help me keep improving and striving for more.

I would also like to thank Dana Chadwick and the SPA lab for helping me with sample processing and data analysis as well as help with methods creation.

Finally, I would like to thank the Undergraduate Research Opportunities Program mini-grant for providing me with the necessary funding for sample processing and field experience.

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## Supplements

**Table 1:** Mixed effects regression results for C:N between species and on and off ant mounds. Block was included as a random effect.

Effects	DF	F-value
species	6, 32	17.79839***
ant	1, 32	5.22664*

~ \*p < .05 ; \*\* .05 < p < .001 ; \*\*\* p < .0001

**Table 2:** Results of pairwise comparisons of C:N between all species.

Contrast	Estimate	SE	df	t-ratio	p-value
<i>Bromus-Hemizonia</i>	42.42	6.5	32	6.528	<.0001
<i>Bromus-Lasthenia</i>	34.03	6.5	32	5.236	0.0002
<i>Bromus-Layia</i>	35.21	6.2	32	5.417	0.0001
<i>Bromus- Lolium</i>	4.03	6.5	32	0.620	0.9956
<i>Bromus- Lotus</i>	47.73	6.5	32	7.344	<.0001
<i>Bromus- Plantago</i>	11.40	6.5	32	1.755	0.5863
<i>Hemizonia-Lasthenia</i>	-8.4	6.5	32	-1.292	0.8504
<i>Hemizonia-Layia</i>	-7.22	6.5	32	-1.11	0.9203
<i>Hemizonia-Lolium</i>	-38.39	6.5	32	-5.908	<.0001
<i>Hemizonia-Lotus</i>	5.3	6.5	32	0.816	0.9815

<i>Hemizonia-Plantago</i>	-31.02	6.5	32	-4.773	0.0007
<i>Lasthenia-Layia</i>	1.18	6.5	32	0.182	1.00
<i>Lasthenia-Lolium</i>	-30.0	6.5	32	-4.615	0.0011
<i>Lasthenia-Lotus</i>	13.70	6.5	32	2.108	0.3724
<i>Lasthenia-Plantago</i>	-22.62	6.5	32	-3.481	0.0222
<i>Layia-Lolium</i>	-31.18	6.5	32	-4.797	0.0006
<i>Layia-Lotus</i>	12.52	6.5	32	1.926	0.4787
<i>Layia-Plantago</i>	-23.8	6.5	32	-3.663	0.0141
<i>Lolium-Lotus</i>	43.7	6.5	32	6.723	<.0001
<i>Lolium-Plantago</i>	7.37	6.5	32	1.134	0.9125
<i>Lotus-Plantago</i>	-36.32	6.5	32	-5.598	0.0001

~ \*p < .05 ; \*\*.05 < p < .001 ; \*\*\* p < .0001

### Shapiro test

We used a Shapiro test function for normality. This function tests whether the null hypothesis, which states that the data are normally distributed, can be rejected with a p-value of .05. The output of this test indicates that the null hypothesis should not be rejected and the data are not normally distributed (w=0.9194, p=0.005784).

### Levene test

We used a Levene test because the data were not normally distributed and it is less sensitive to departures from normality. This function tests the equality of variances for a variable calculated for two or more groups and is rejected with a p-value of .05. The output of this test indicates that the null hypothesis should be rejected and the data are homoscedastic in variance (F value: 0.6875, p-value: 0.7585).