Title

Nipomo Lupine (Lupinus nipomensis) 2019-20 Year Outplanting and Assessment Studies

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During the 2019-20 hydrologic year several projects were initiated which are designed to evaluate questions resulting from the long-term Black Lake Ecological Area (BLEA) Nipomo lupine outplanting experiment. These include an assessment of soil conditions at various sites, an assessment of soil moisture at multiple sites and two additional experimental outplanting trials to evaluate the extent to which fog collection by cages could be more of a factor than herbivory-protection and to evaluate whether there are any benefits to lupine survival from growing with versus without other plants nearby.

Experimental Outplanting Trials:

Two questions came up in discussions with the recovery team regarding the results of the initial outplanting experiments from BLEA from 2014-15 and 2015-16 which were: 1. Could the uncaged plots where lupine seeds were vulnerable to herbivory have been negatively impacted by the fact that there was no mesh above the seedlings that could potentially capture fog moisture? 2. Some lupine seedlings have been observed germinating within the shade or immediately adjacent to veldt grass and other potential competitors. Could there be a facilitative function for other plants? To address these questions two trials were designed. The first is called "Fog versus Herbivory" which essentially creates an opportunity for lupine seedlings to receive potential additional of fog dripping off of a meshed enclosure (e.g. a cage) but one that is lifted off the ground to allow for herbivory. This will help demonstrate whether the lack of seedlings in the uncaged treatment from earlier studies was due to herbivory or a differential soil moisture condition. The second experiment designed protects all plants from herbivory in cages but evaluates competition by placing seeds in completely cleared and weeded plots and other seeds within a matrix of existing vegetation where weeds will not be pulled. This could help address whether lupine germination, growth and seed production benefit from some intermittent clearing or disturbance to open the site and whether competition and shading impact the species.

The "fog versus herbivory" experiment was established at Black Lake Ecological Area (BLEA) and included five pairs of caged plots in three topographic positions (south facing, north facing and swale) in which 1 cage in each pair was lifted 5 inches off the ground to allow for small mammal/bird herbivory while retaining the cage to potentially allow for fog drip from cage mesh tops (Figure 1, Map; Figure 2 photos).

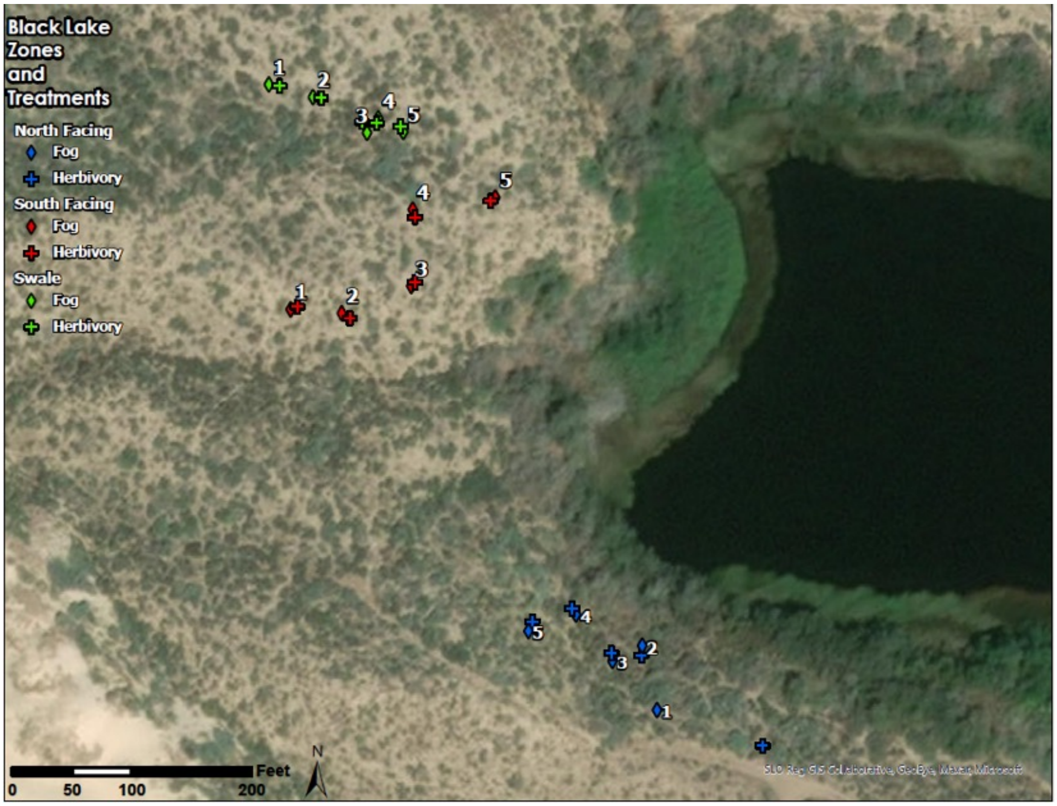


Figure 1. Map of Fog vs Herbivory Trial at Black Lake Ecological Reserve.

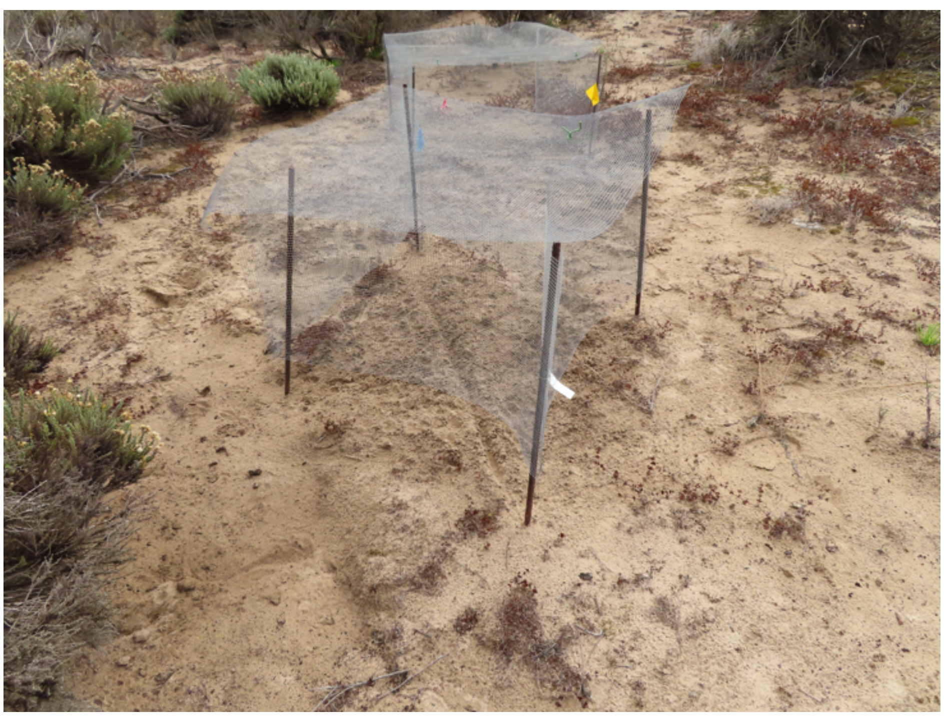


Figure 2a. Raised cage. Collects fog and allows for herbivory.

Figure 2b. Fully caged to collect fog and prevent herbivory.

The second 2019-20 experimental trial is the competition/facilitation experiment which was conducted in the swale at Kathleen Goddard Jones Overlook (KGJ Overlook) site where 5 pairs of cages were established at three topographic positions (south facing, north facing and swale sites). One of each pair of cages was fully weeded with a one-meter buffer around the cage (of either *Ehrharta calycina* (on south facing slope) or native plants (swale and north facing slopes) and the other was established within a matrix of non-native grass or native shrubs.

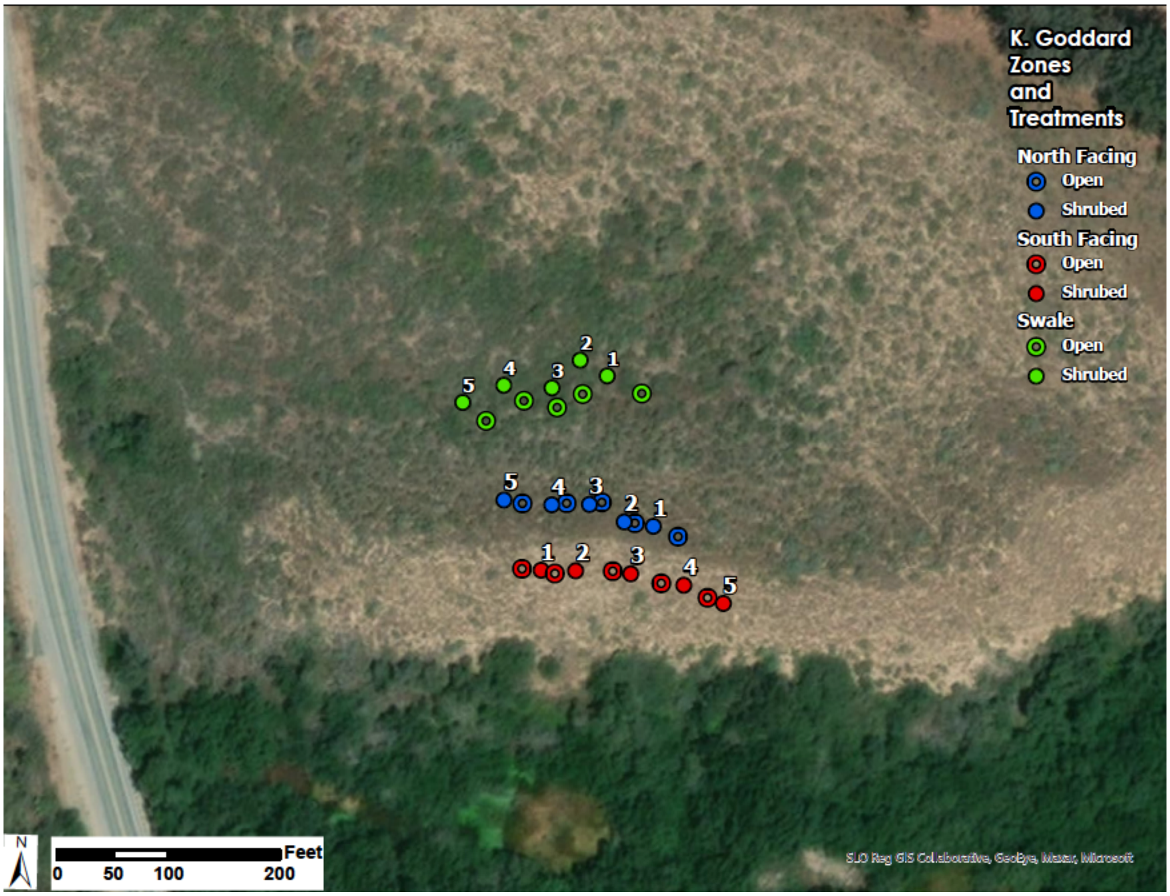


Figure 3. Experimental plots at Kathleen Goddard Jones Overlook comparing with vegetated, un-weeded (+ competition, “shrubbed”) with cleared and regularly weeded (- competition, “open”) treatments within caged plots.

In December 2019 we planted 50 seeds (25 scarified and 25 unscarified), in each cage in both trials, however there was essentially no germination in either trial because seeds had very low viability. Seeds used in 2019 were stored in jars from the 2016 bulking effort before they were fully dry (Chapman 2020 Seed Germination Report). In winter 2020, after a concerted greenhouse effort, we had less than 3% of seeds from the 2016 bulking effort germinate in the greenhouse despite multiple pre-treatments, so the lack of field germination in the experimental trials is not surprising. In addition, the 2019-20 rainfall year was not very conducive to lupine growth since there was a 2.5 month hiatus in rain from early January to mid-March 2020 which provides challenging conditions for this annual plant. Nevertheless, lessons were learned from the caged treatments that are informing the 2020-21 trial year.

On November 18th, 2020, the two trials were re-seeded with viable seeds (20 scarified and 20 unscarified seeds per cage) for a total of 1200 seeds per trail. Scarified seeds were placed on the south side of a diagonal line drawn between the SW and the NE corner of each plot.

Lessons learned from 2019-20 Trials.

In June cover was assessed in all the BLEA plots for all species (except the Nipomo lupine) and the average height of each species was documented. In a subset of plots, the biomass of all natives and all non-natives were collected in two paper bags and oven dried at 65 C. The correlation between the estimated cover times the height for all natives and for the non-natives was summed and correlated to the biomass. The correlations were fairly high so we show data for biomass in the graphs below:

Native biomass = 0.0485*(height)*(percent cover) + 10.3554 , $R^2 = 0.7996$

Nonnative biomass = 0.03718*(height)*(percent cover) + 6.85042 , $R^2 = 0.5752$

Information drawn from cover and biomass estimates were used to characterize overall growing conditions in the different treatments.

We observed that south facing slopes had lower plant biomass and total cover than north-facing slopes and swales in the BLEA 2019-20 Fog versus Herbivory trial (figure 4a&b). The raised cage (potential for herbivory treatment) had lower plant cover and biomass than the grounded (anti-herbivory) treatments in the north facing and swale sites where there was enough biomass to show an effect. This indicates that herbivory does play a role for dune species and likely affects Nipomo lupine (Figure 5a and b). Interestingly, the north facing slope plots had the highest proportion of non-native cover, made up primarily of annual grasses like *Bromus diandrus* (Figure 6).

2020-21 Monitoring and Weeding Plans

The lessons that were learned include that there was significant growth of non-lupine species in all the trials and so in the 2020-21 year we will be weeding the KG Jones trial plots designated as the "open" or "no competition" treatment through the growing season and will leave all plants in the +competition treatment plots ("shrubbed"). The KG Jones "open (–competition)" plots will be weeded on a biweekly basis to compare them to the (+competition) treatment plots. In the BLEA plots, only veldt grass and slender iceplant will be weeded from any plots. All plots, except the competitive KG Jones plots, were fully cleared during the fall 2020 to provide an open surface for lupine germination.

All trials will be monitored in the 2020-21 year every two weeks for lupine germination, survivorship and reproductive output. Late in the growing season, but before plants dry up, cover and average height of all non-lupine species will be estimated for each plot.

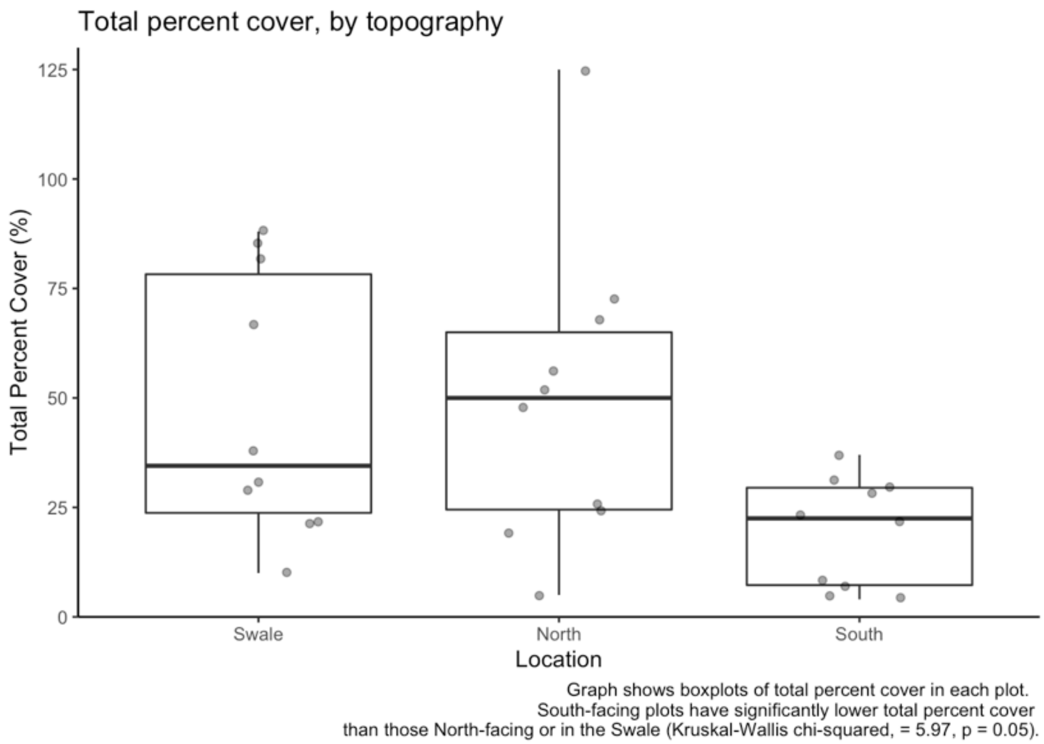


Figure 4a. Total cover of non-lupine species in 2019-20 BLEA Fog/Herbivory Trial between slope aspect positions. South facing has significantly lower percent cover.

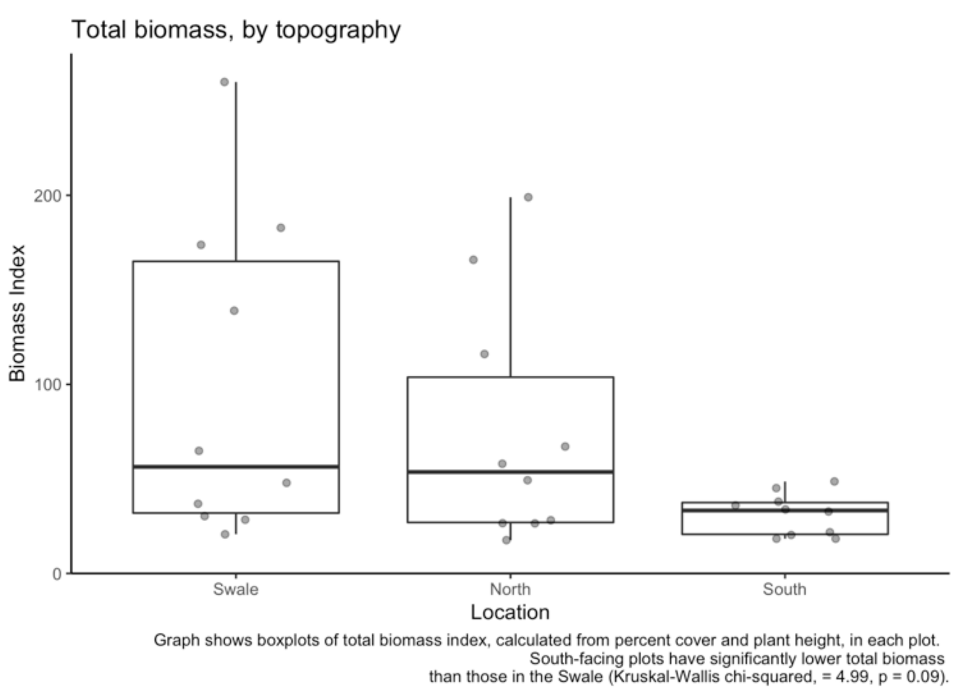


Figure 4b. Biomass calculations of the trial confirm that north-facing and swale sites support higher plant biomass and cover than south facing slopes.

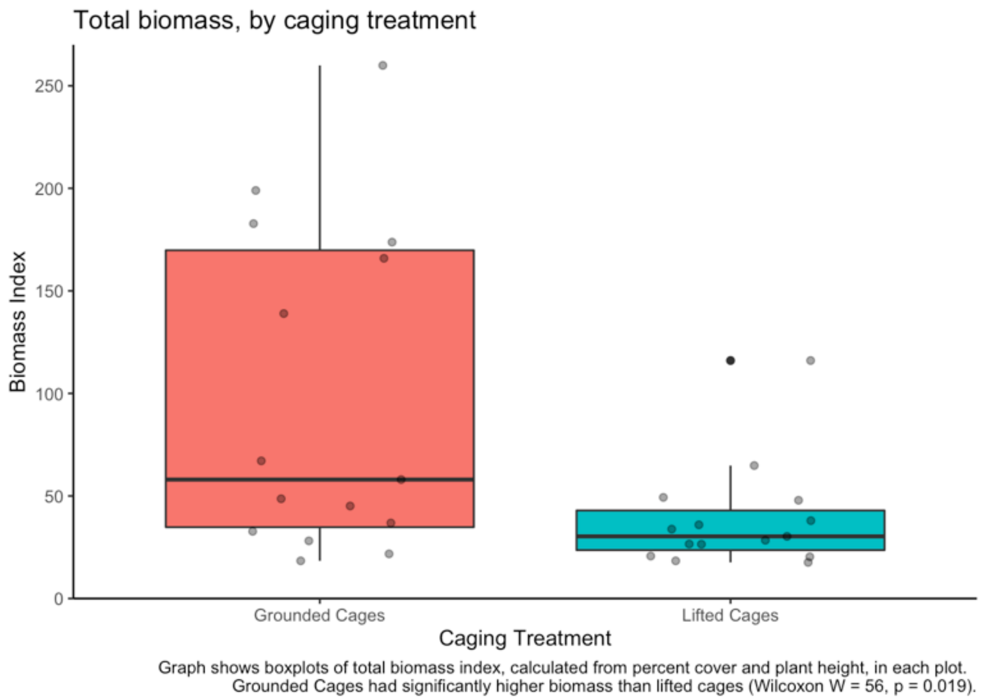


Figure 5a. Lifted cages in the Fog/Herbivory trial expose plants to herbivory and show lower total biomass, and cover (not shown).

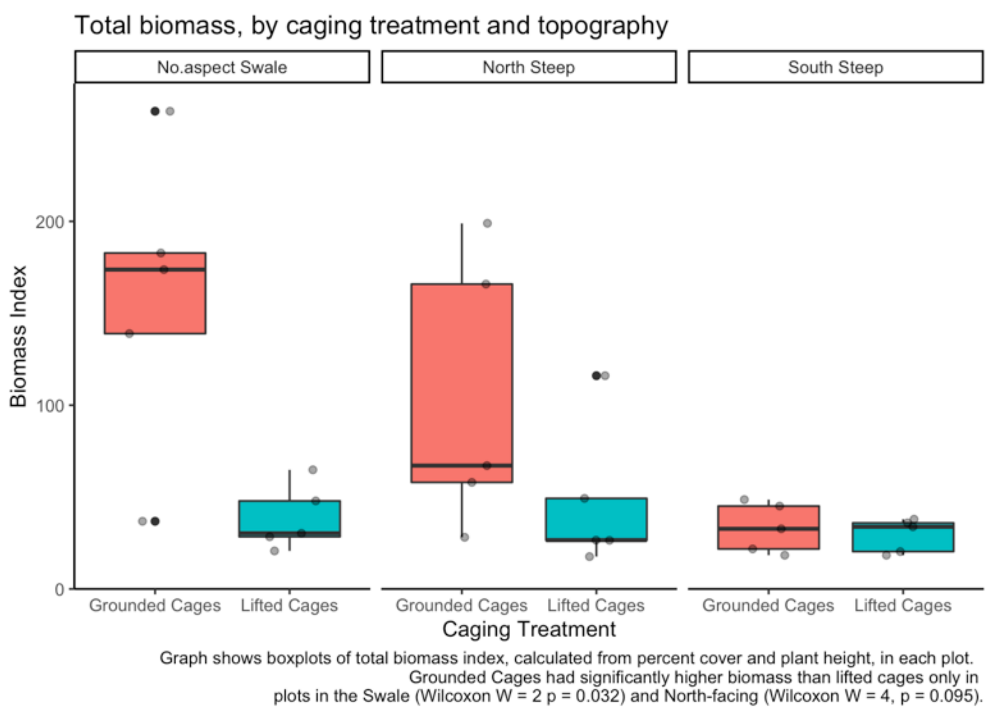
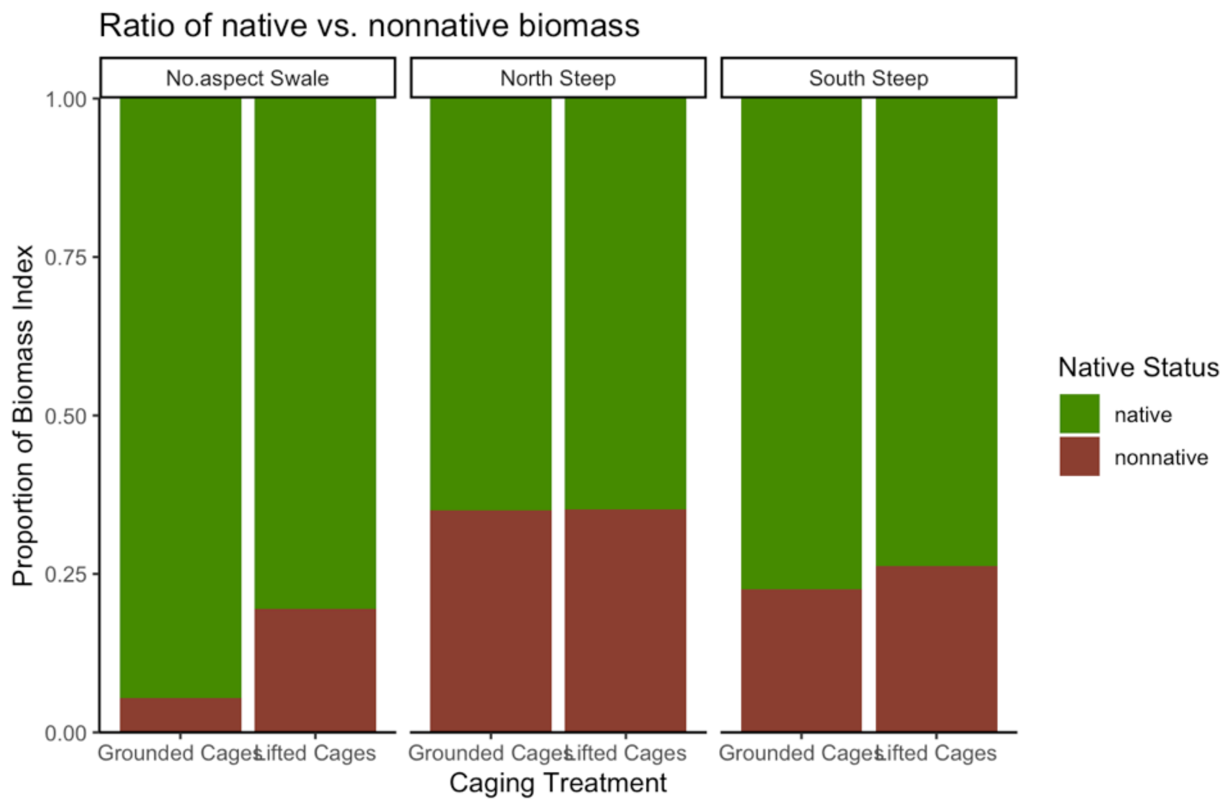


Figure 5b. The higher the biomass, the more significant is the effect of the caging treatment on total biomass.



n of native and nonnative biomass index (biomass index calculated from percent cover and plant height).
Significantly higher proportion of nonnative biomass in the North-facing plots
than that in the the Swale plots (Kruskal-Wallis chi-squared = 10.43, p = 0.005).

Figure 6. Relative proportion of native and non-native biomass by slope and aspect in BLEA 2019 Trial.

Cover comparison for BLEA 2014-2015 Plots

Similar results were found for cover and biomass of non-lupine plants in the 2014-15 and 2015-16 plots. Cover and biomass within caged plots was higher than in uncaged plots and cover in the south facing sites was lower than in the other aspects. Interestingly the ridge sites had higher cover than the north facing slopes but not higher than the swale sites.

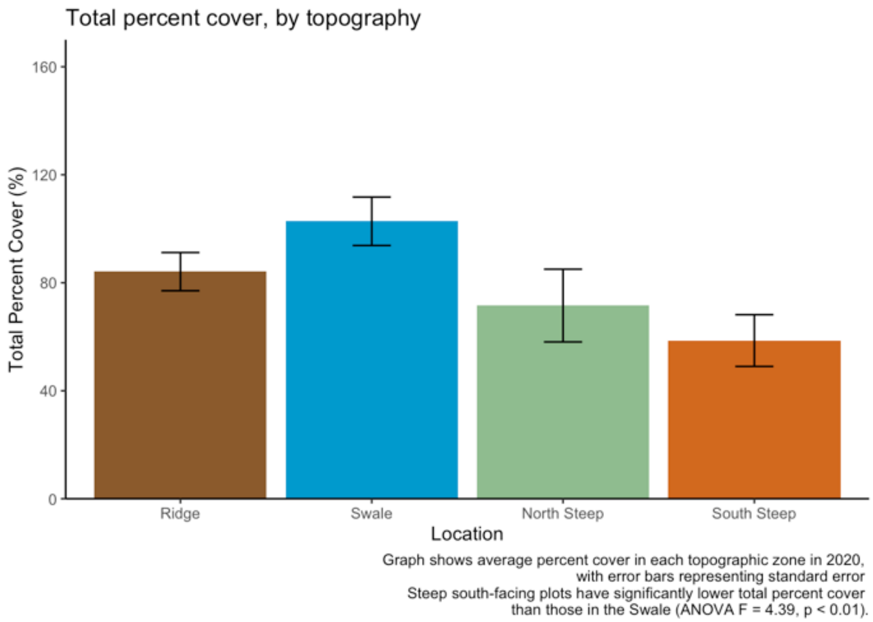
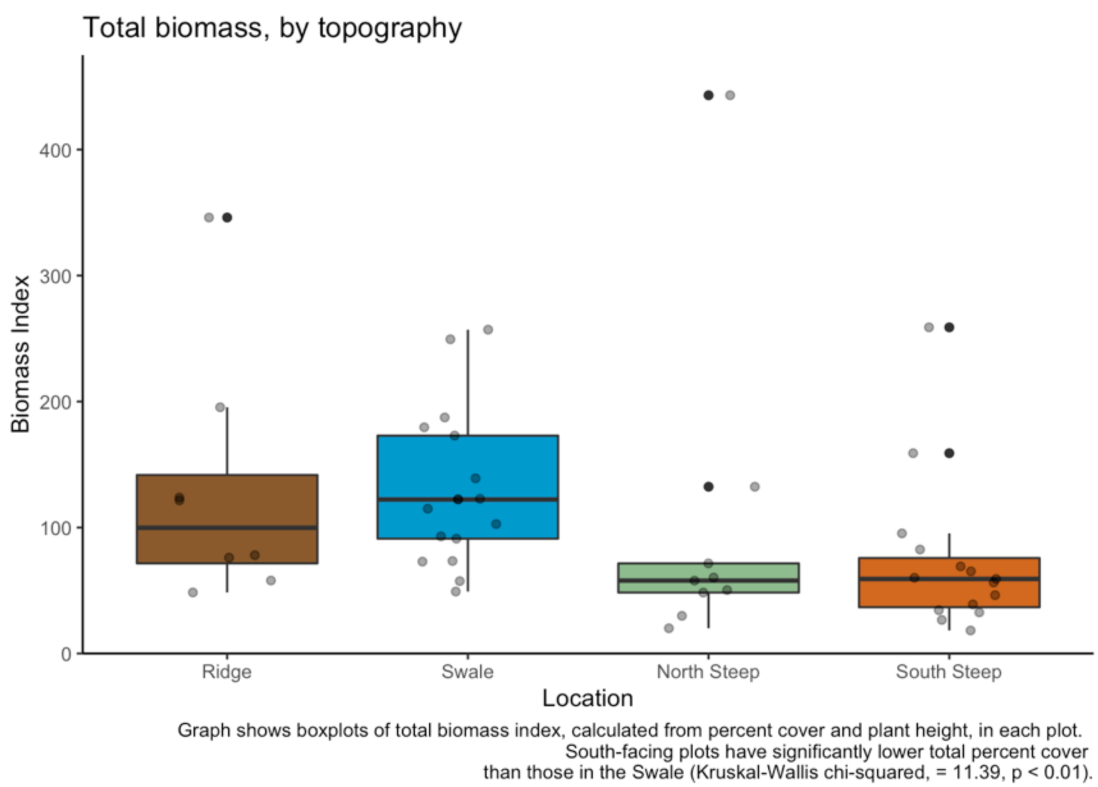


Figure 7a. Cover of species aside from *Lupinus nipomensis* by topographic position



7b. Biomass by topography shows a similar pattern of the swale having the highest biomass.

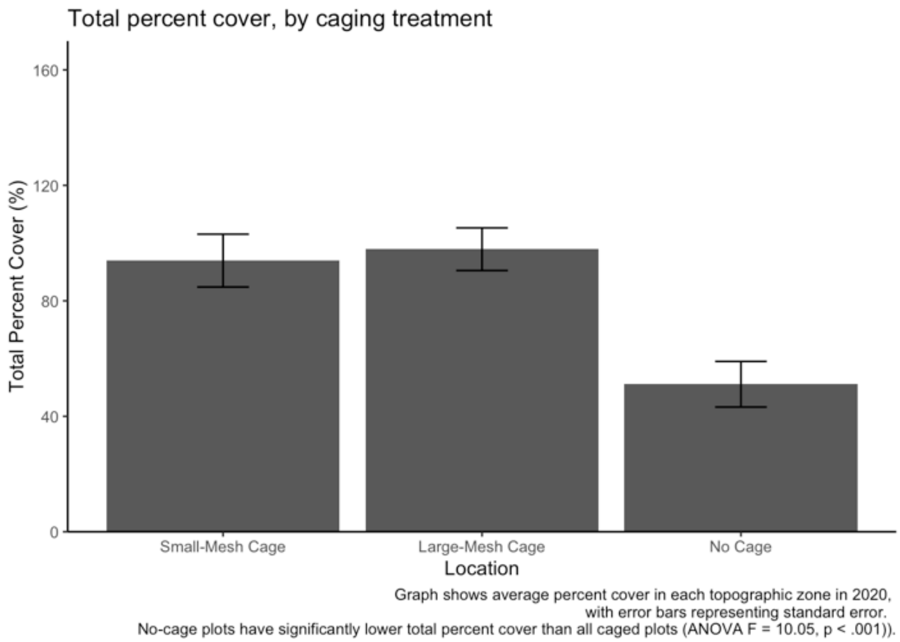
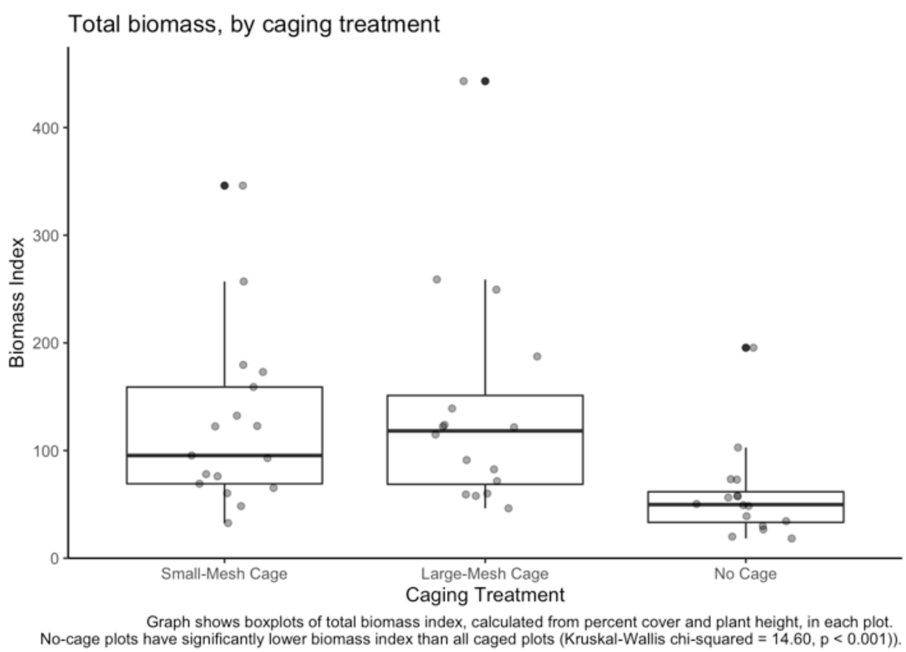
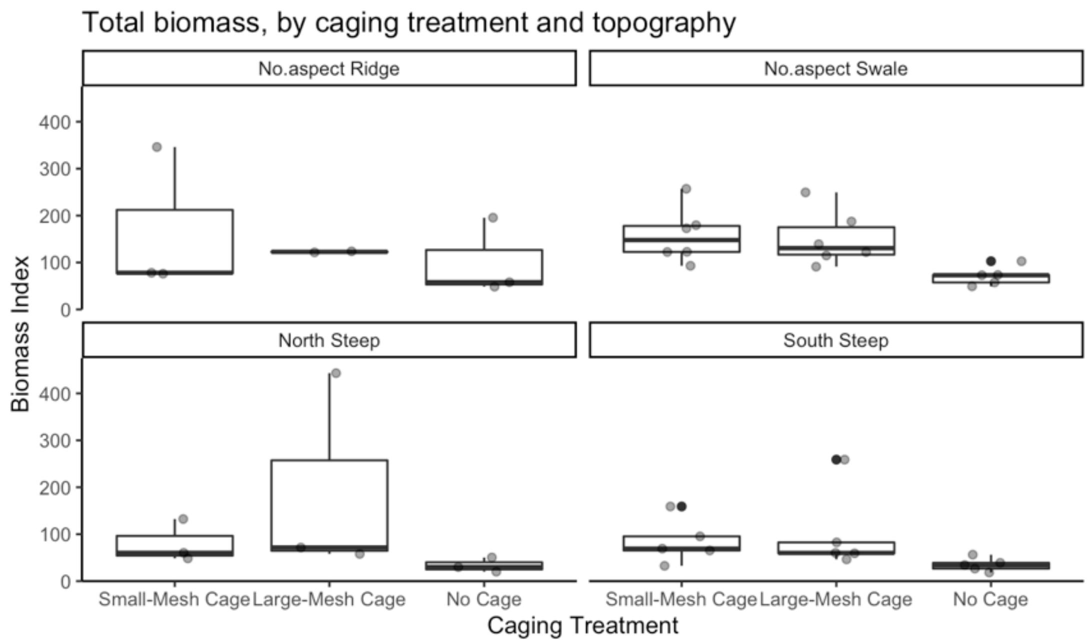


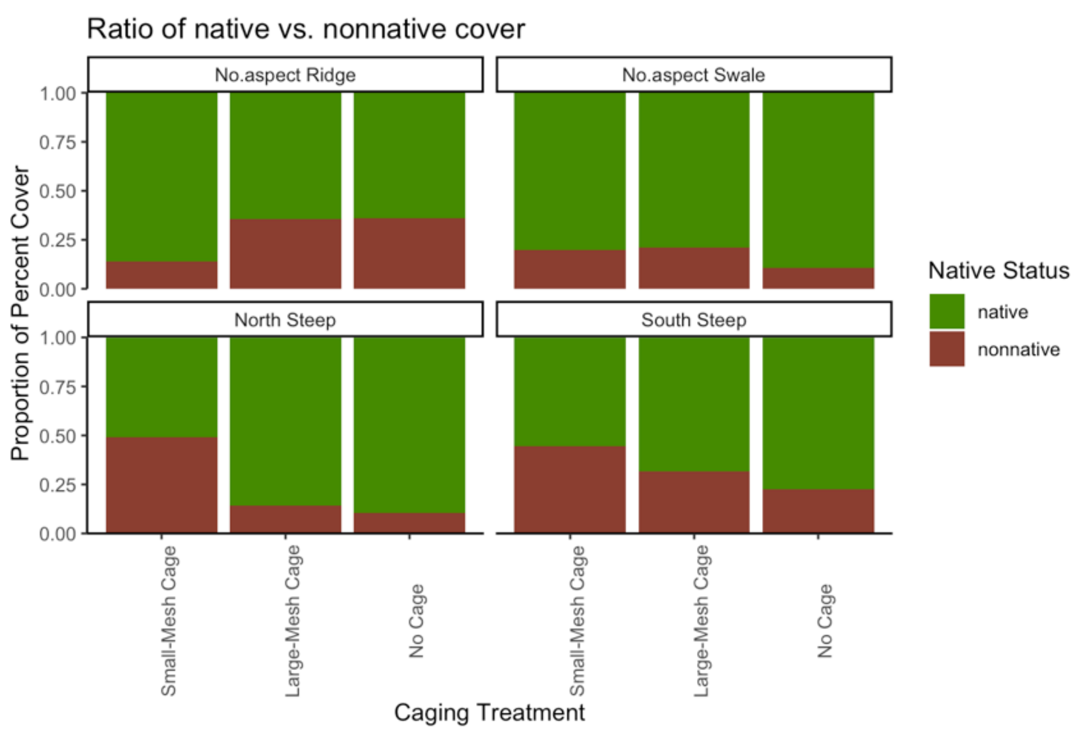
Figure 8a. Percent cover by caging treatment.



8b. Total biomass by caging treatment shows similar trend of lower vegetation in the uncaged treatment plots.



Graph shows boxplots of total biomass index, calculated from percent cover and plant height, in each plot.
No-cage plots only had significantly lower biomass in
Swale compared to Large-mesh Cage plots (Tukey's post-hoc p = 0.03)
and Small-mesh Cage plots (Tukey's post-hoc p = 0.02),
and in South Steep plots compared to Large-mesh Cage plots (Tukey's post-hoc p = 0.06)
and Small-mesh Cage plots (Tukey's post-hoc p = 0.07).



Graph shows mean proportion of native and nonnative percent cover.
No significant differences in ratio of native:nonnative cover (Kruskal-Wallis chi-squared = 5.69, p = 0.13).

Figure 9. There was no significant effect on the proportion of native to non-native by topographic position or cage type.

Abiotic Conditions Study

Data was taken to better characterize the abiotic conditions of the different topographical sites in the BLEA long-term experimental outplanting site by assessing soil moisture using Time Domain Reflectometry (TDR) techniques to deliver volumetric water content (Field Scout, TDR 300, Spectrum Technologies). In addition, soil samples (surface, 50 cm, 100 cm and 150 cm in depth) were collected from both the existing extant populations in the Dune Protected Area (DPA) on Phillips 66 land and the Black Lake and Kathleen Goddard Jones outplanting sites considered most likely to be successful. Soil samples were analyzed for soil texture, moisture (gravimetric), pH, EC, and % organic matter using a muffle furnace by FGL Environmental (Fruit Growers Lab).

Soil moisture, % silt, and % organic matter were highest in swale sites (Kathleen Goddard Jones Overlook site was collected from a swale and "Swale 5" is the swale site in BLEA) (Figures 10 and 11(a-e)). The reference site, Philips 66, was generally lower in silt and had comparable or lower, soil moisture, organic matter, EC and pH, relative to all existing and proposed outplanting sites. These data were exploratory and only included one sample from each site that was sent to the lab. Further replication could be conducted if the results warrant a closer examination. The soil moisture results suggest that the soil moisture overall is low but is higher in the swale site at BLEA than the slope sites.

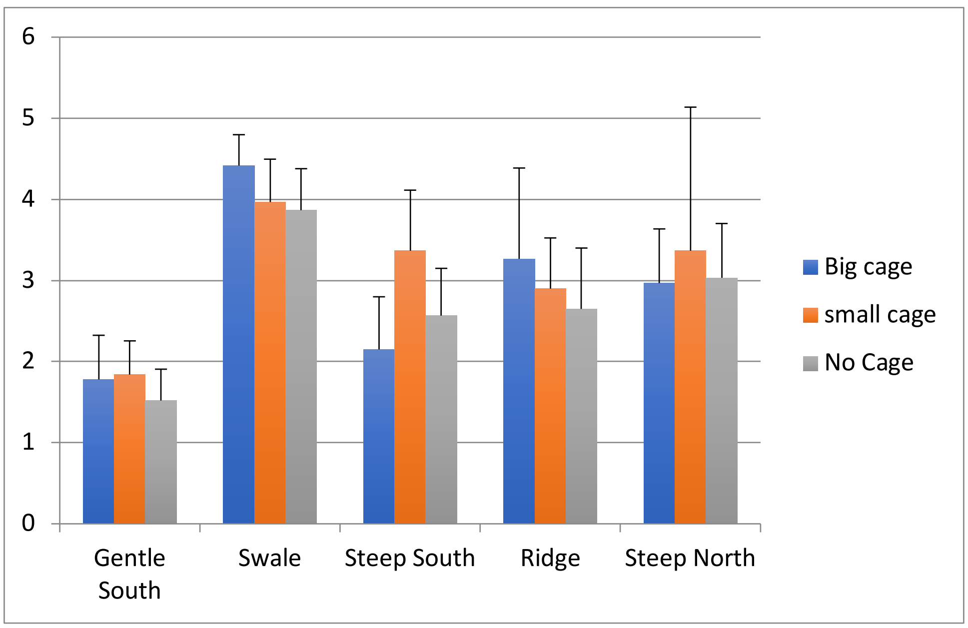


Figure 10. Volumetric soil moisture by topographic site and cage type in the BLEA 2014-15 trial.

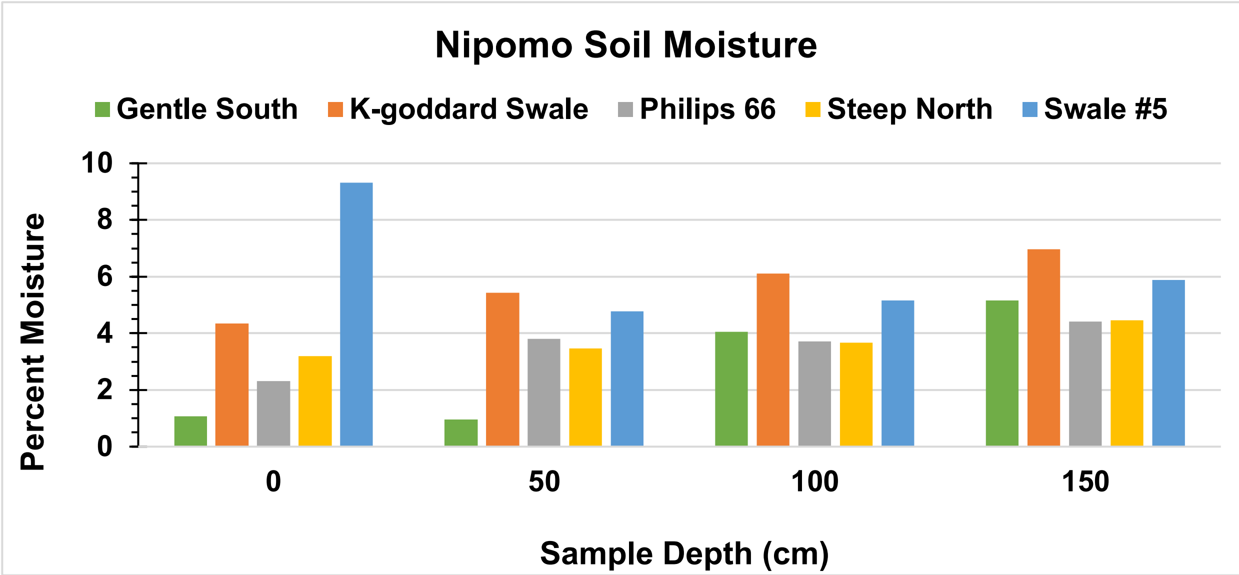


Figure 11a. Gravimetric soil moisture by depth and site. Gentle south, steep north and swale # 5 occur at Black Lake Ecological Area; K-goddard swale is at the swale site of the Kathleen Goddard Jones Overlook area and Philips 66 is the location of the extant population in the Dune Protected Area (DPA).

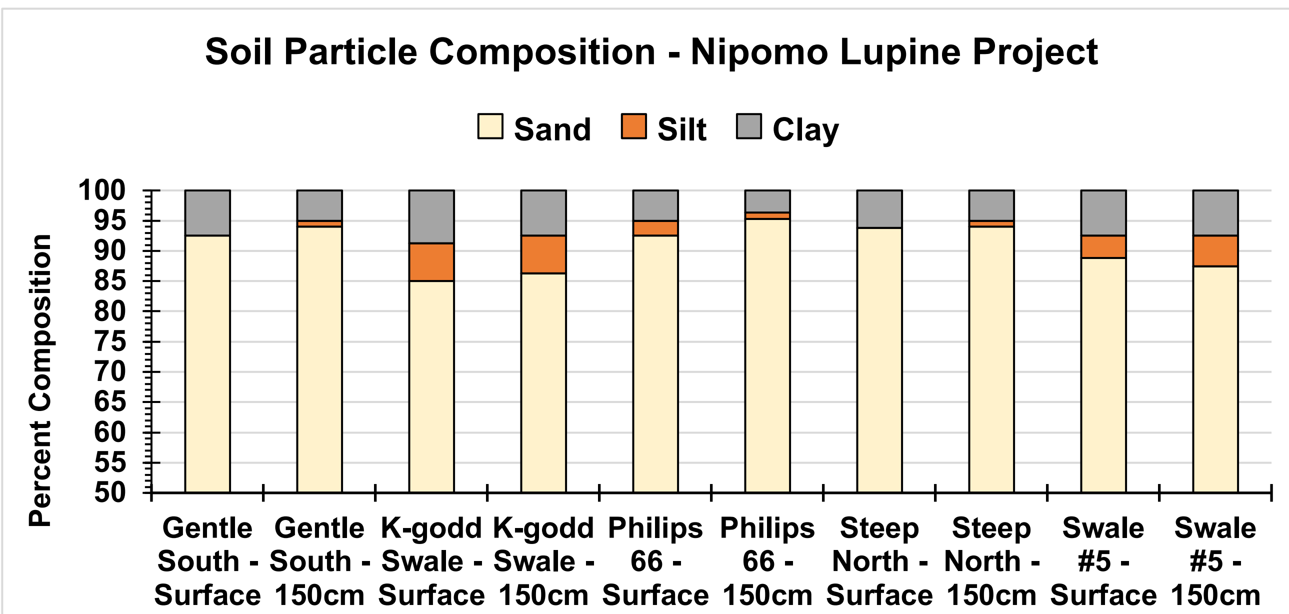


Figure 11b. Soil texture based on hydrometer methods at Fruit Growers Lab for surface and at 150 cm depth.

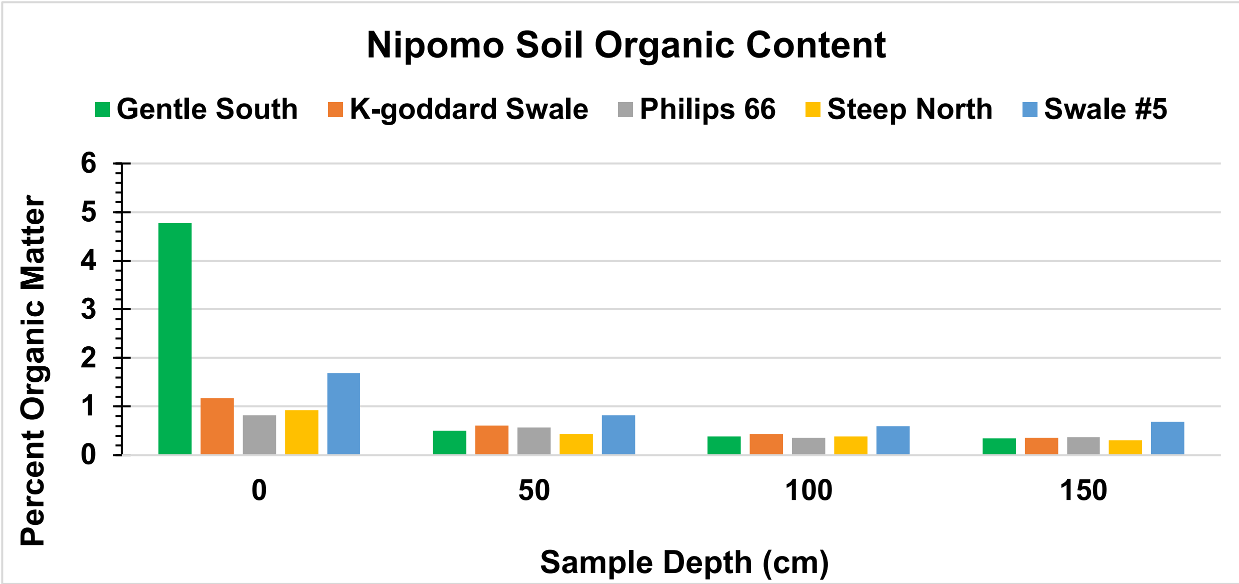


Figure 11c. Soil organic content based on muffle furnace methods by depth and site. Gentle South site has significant Eucalyptus duff which may explain high organic matter content in the surface sample of that site.

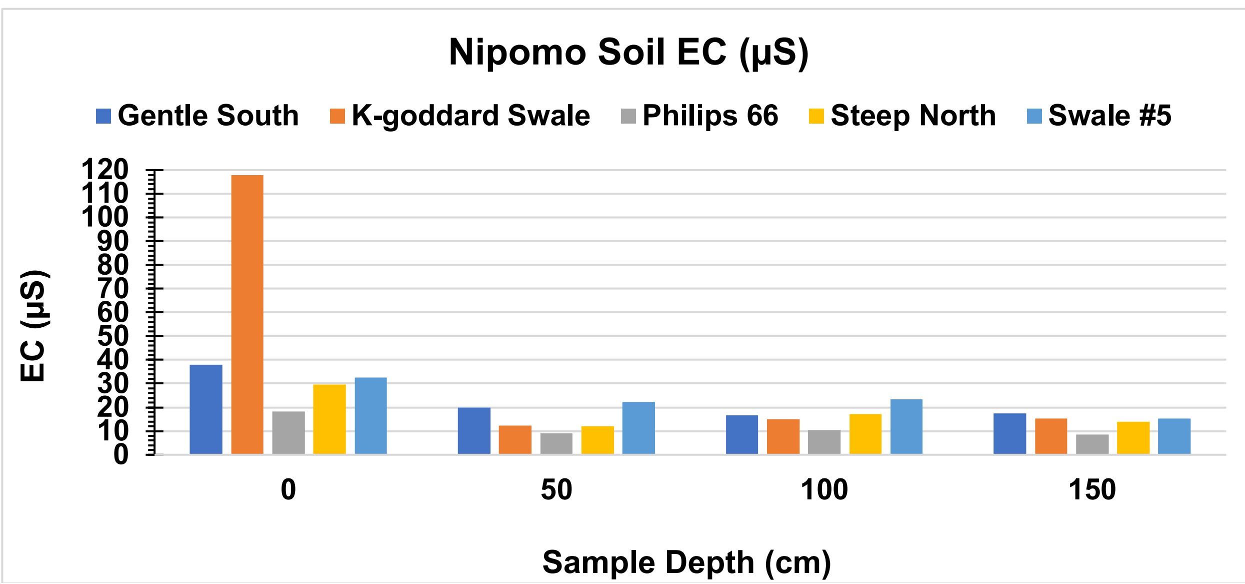


Figure 11d. Soil electrical conductivity (micro Siemens/cm) by site and depth.

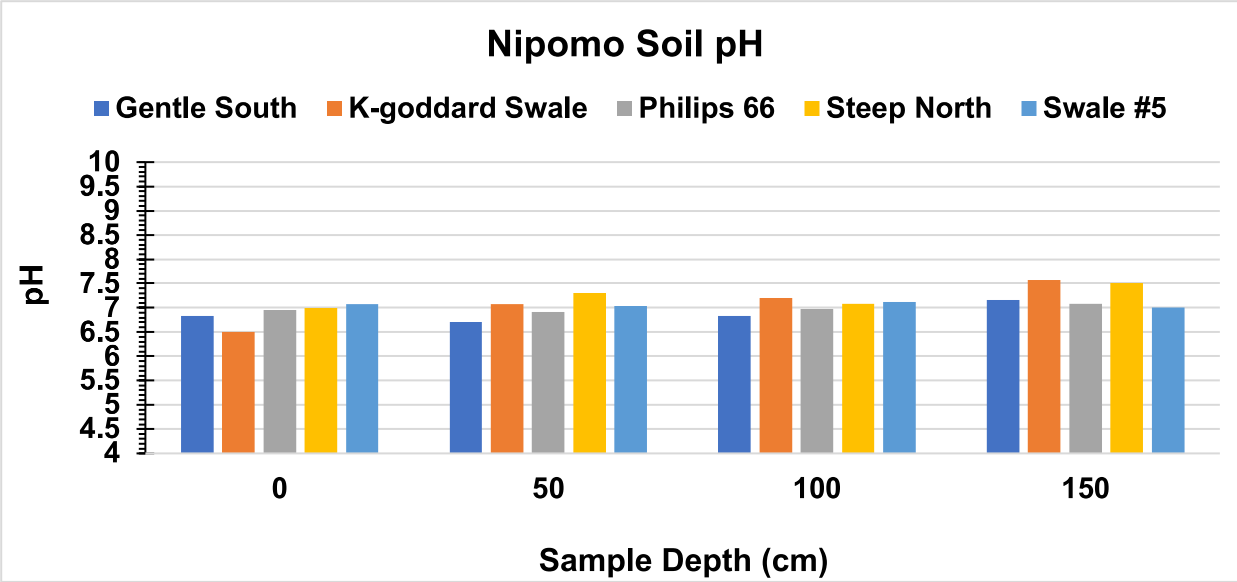


Figure 11e. Soil pH by site and depth.