

Nest Distribution of *Bombus* spp. Across Different Habitat Types

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Abstract

Despite bumble bees' critical importance as pollinators throughout the montane regions of Colorado, little research has been done on their nesting habits. In order to conserve the populations of *Bombus* spp. in the West, better knowledge of their nest density and nesting habitats is needed. I hypothesized that (1) a nest searching technique would be successful, (2) *Bombus* spp. nests would be identified in three distinct habitats, and (3) there would be a difference in nest densities across habitats depending on the bumble bee species. The habitats that I searched were classified as wet meadow, dry meadow, and aspen forest. Across three separate sites, each habitat had a total of 9 plots. Each of these plots was 150 square meters. I spent 30 minutes at each site once a week searching for nests and *Bombus* activity. Results of this study showed success of the nest searching technique and identification of *Bombus* nests in all three habitat types, but there was insufficient data to form a conclusion regarding the nesting habitat preferences of different species. The conclusion of this study is that dry meadow has the highest nesting density and should be preserved. Because this habitat also functions as foraging habitat for *Bombus* species, the conservation of this land is beneficial for the whole life cycle of the pollinators

Introduction***Background***

Despite their importance as pollinators, both in agriculture and natural environments, populations of bumble bee species have been steadily declining in North America (Cameron & Sadd, 2020). In the montane regions of the West, bumble bees are important pollinators for the many species of wildflowers present. The different proboscis lengths of different species of bumble bee allows

for generalist and specialist species and unique pollinator-plant interactions (Cariveau et al., 2016). This means that with the decrease in population size of certain *Bombus* species in the montane regions of Colorado, there are many environmental consequences including biodiversity loss (Soroye et al., 2020).

This decline in bumble bee populations is due largely to environmental factors such as climate change and habitat loss which lead to a lack of floral resources and loss of habitat and nesting materials (Hines & Hendrix, 2005; Soroye et al., 2020; Graves et al., 2020). Lack of floral resources is often considered the most detrimental factor to *Bombus* colony survival and reproductive output (Potts et al., 2010), but habitat loss and lack of nesting materials is equally if not more catastrophic. Regardless of the abundance of floral resources such as pollen and nectar, without a proper nest no colony can form.

As there is more knowledge surrounding the floral resource availability for bumble bee colonies, most efforts are centered around the conservation of floral environments (Goulson et al., 2007). Though the foraging habitats of bumble bees are a necessary resource for their survival, the presence of nesting habitat is the basis for a colony. Due to the lack of understanding surrounding *Bombus* nesting habits, including in which habitat types they nest, conserving the nesting habitat of bumble bees is extremely difficult.

Research Questions, Goals, and Hypothesis

Put simply, this research aimed to answer the question of where *Bombus* spp. nest. The goal of this study was to determine the nest density and habitat selection of *Bombus* spp. in the montane regions of Colorado using the methods outlined in Pugeseck & Crone (2021). Using these methods, this study aimed to answer three main questions: (1) Do the methods outlined in

Pugesek & Crone (2021) work in the montane regions of Colorado? (2) What are the nest densities of different *Bombus* spp. across three distinct habitat types? (3) Do different species prefer different habitats?

My hypotheses for this study were that (1) the methods from Pugesek & Crone will be successful in nest searching efforts in the west, (2) *Bombus* nests will be identified in all three habitats in different densities, and (3) different species of *Bombus* will have different nesting habitat preferences.

Methods

Study Sites

The study was carried out across three sites (Figures 1-4). The sites were generally similar and all had the same three distinct habitats: dry meadow, wet meadow, and aspen forest. Dry meadow was described as having less vegetation and more exposed ground than much of the surrounding area while wet meadow had much more lush vegetation and less exposed ground. The aspen forest habitat was any kind of vegetation within an aspen forest. Each habitat had six 150 square meter plots across the three sites for a total of 18 plots across three sites and three habitat types. All habitat types were relatively open and had near equal access to floral resources, ensuring that floral abundance would not affect the nest habitat preferences of the bees.

Nest Searching

Each plot was visited and thoroughly searched for nests for 30 minutes once a week. When possible, visits occurred between the hours of 8:00 am and 6:00 pm when the weather was relatively warm and clear. I walked slowly through each transect searching for any *Bombus*

activity that may indicate a nest. If I identified a bee, I followed its foraging behavior, sometimes resulting in the discovery of a nest. When a nest was identified within a plot, I placed a colored flag about 10 centimeters to any side of the nest entrance as to not disturb or call any unwanted attention to the colony. I paused and recorded information about it. This included bumble bee species (*B. bifarius*, *B. occidentalis*, etc.), location (below ground, ground surface, above ground), substrate (dirt, grass, dead tree, etc.) habitat (dry meadow, wet meadow, aspen forest), and any other relevant information. I then resumed nest searching until the 30 minutes were over.

Recording Nest Location

Before I began collecting data and nest searching, I visited the sites and recorded the locations of the plots using a GeoXT Terrasync. I revisited the sites with the Terrasync outside of the 30 minutes of nest searching per transect in order to record the presence and location of nests. Each nest was represented by a location point. This was used for reference and data analysis.

Statistical Analysis

Results from this study were analyzed using nest density. Nest densities, as a decimal, were found by dividing the number of nests in each habitat by 900 (the total area of a habitat across three sites). The differences in nest densities were also found. This was done in R using a non-parametric Kruskal Wallis test. A Wilcox test was used to determine any differences between habitats. The data was then graphed into a box plot and further split into three bar graphs based on habitat, location, and substrate.

Results

Research Question and Hypotheses

The research question of this study was broken down into three sub-questions. These questions asked (1) Do the methods outlined in Pugesek & Crone (2021) work in the montane regions of Colorado? (2) What are the nest densities of different *Bombus* spp. across three distinct habitat types? (3) Do different species prefer different habitats? The hypotheses for these questions stated that (1) the methods from Pugesek & Crone will be successful in nest searching efforts in the west, (2) *Bombus* nests will be identified in all three habitats in different densities, and (3) different species of *Bombus* will have different nesting habitat preferences.

Hypothesis Results

Two of the three hypotheses of this study were supported. The first hypothesis, stating that the methods from Pugesek & Crone (2021) would be successful, was supported. Of 54 observations, 18 were successful in identifying nests across different habitats (Figure 5). This was done using the methods from Pugesek & Crone (2021).

The second hypothesis of this study, stating that *Bombus* nests were identified in all three habitats, was also supported. Of 18 identified nests, 12 were found in dry meadow, 5 in wet meadow, and 1 in aspen (Figure 6). These results showed nest densities of 0.0133, 0.0055, and 0.0011 per m². The differences between these nest densities were analyzed in R using a non-parametric Kruskal Wallis test. This test compared the number of nests found in each habitat across all observations and found that the nest numbers significantly varied across habitats ($P = 0.004181$). A Wilcox test was then used to determine the differences between habits. This test in showed that dry meadow and aspen were different in the number of nests ($P = 0.0052$), through

the number of nests did not significantly differ between both wet meadow and dry meadow, and wet meadow and aspen forest habitats ($P > 0.05$).

The third hypothesis of this study was not supported as there was insufficient data to form any conclusions. Although some bees seemed to show a preference for one habitat over another (Figure 7), there was simply not enough data to conclude anything in terms of preference.

Other Results

Along with habitat preference, location relative to the ground surface and substrate preferences were also analyzed. Of 18 nests, 12 were identified below ground while 6 were identified on the ground surface (Figure 8). For substrate preference, of the 18 nests 6 were in bunch grass, 4 in ground holes, 7 in roots, and 1 in sagebrush (Figure 9). There were no obvious preferences for either location or substrate.

Discussion

Hypothesis Results

The first hypothesis of this study was supported. This means that the methods from Pugsek & Crone (2021) were successful in nest searching efforts in the montane regions of the West. The results showed sufficient evidence that these methods *are* successful.

The second hypothesis of this study was supported as well. *Bombus* nests *were* identified in all three habitat types in different densities. The number of nests differed across habitats. In particular, the number of nests found in the dry meadow was higher than in the aspen forest, but the remaining comparisons were not different.

Comparisons to Pugesek & Crone (2021)

The results of this study showed that most *Bombus* nests were identified in meadow habitats with very few found in aspen. This is relatively consistent with the results from Pugesek & Crone (2021). In 2019, Pugesek & Crone (2021) found the majority of nests in meadow habitats as opposed to hay fields or forests. Our location relative to the surface of the ground was also consistent with results from Pugesek & Crone (2021). They found many nests underground with a number found on the ground surface. Our study also found most nests below ground with a number on the ground surface.

Differences in Expected Results

Although we hypothesized that there would be different nest densities across different habitat types, it was a surprise to see such vast differences from dry meadow to aspen. Based on nest searching behavior data from Dr. Jane Ogilvie, we expected to see slight differences but overall similar nest densities across the three habitats. Dr. Ogilvie has observed that there are differences between species, but on a community level *Bombus* queens nest search relatively equally across the three habitat types.

Possible Sources of Error

The most likely source of error that may have occurred in this study is detection bias. As shown in Pugesek & Crone (2021) there is a detection bias towards dry meadow habitats where most ground is exposed, so it may be easier to detect bees entering and exiting nests. This makes sense as most of our observed nests were under the ground surface and accessed by a hole or root system beneath a plant.

Conservation

The main goal of this study was to improve knowledge of nesting habitat for conservation purposes. The findings of this paper suggest that there is considerable overlap between nesting and foraging habitats for bumble bees. This means that only one main habitat type is required for conservation in order to support *Bombus* populations. The conservation of wet and dry meadow habitats will support all stages of these pollinator's life cycles (Murray et al., 2009).

In areas such as the Gunnison Valley, especially near the Rocky Mountain Biological Laboratory, this kind of habitat is already conserved to an extent, but there's more that can be done (Wright et al., 2017). Other landscapes, especially those being rapidly urbanized, on the other hand, are in desperate need of conservation efforts. This study provides evidence that conserving meadow habitat is the best way to conserve bumble bee populations in montane regions of the West.

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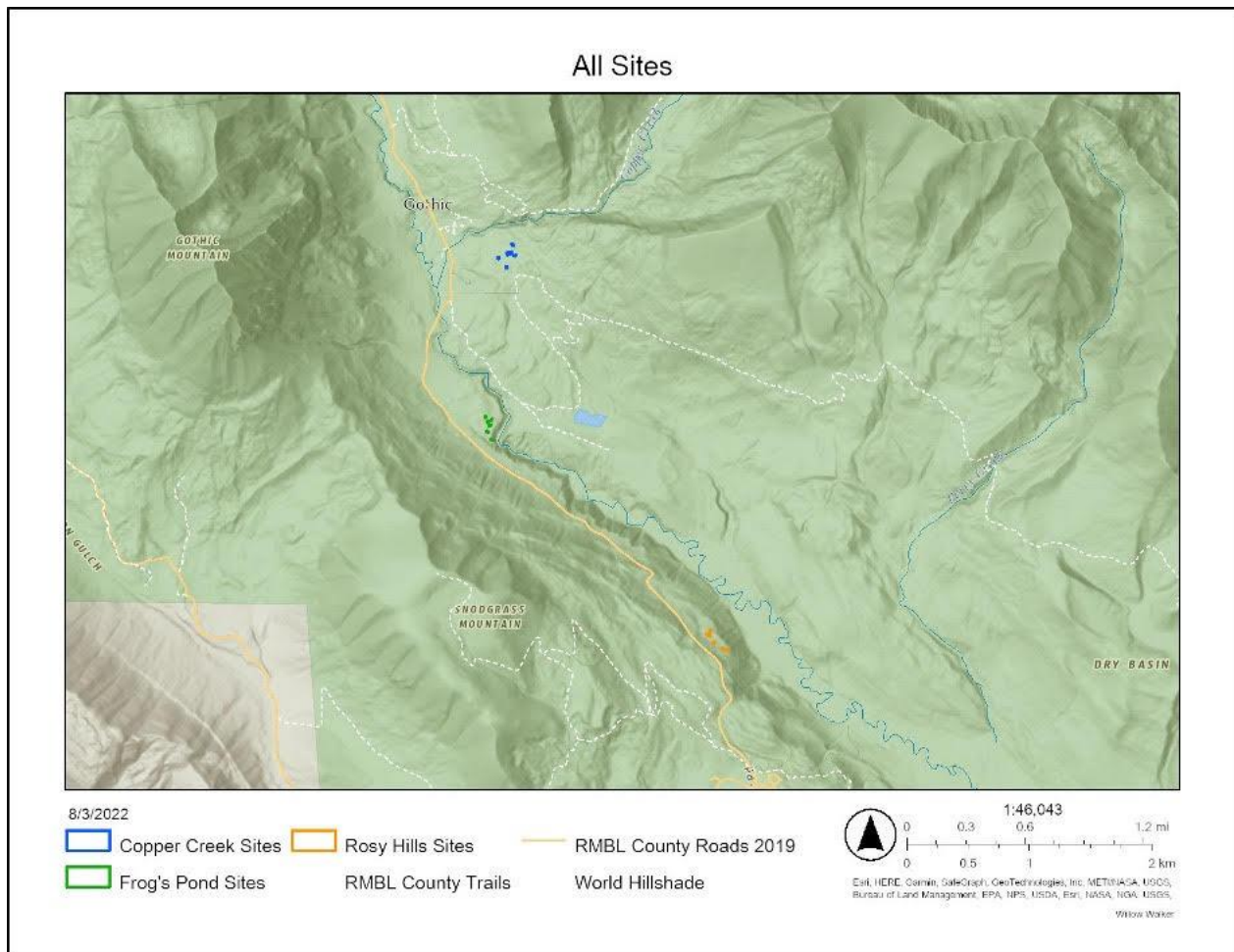
Figures**Maps**

Fig. 1. This figure shows a map of all three sites in the townsite. Copper Creek plots are shown in blue, Frog's Pond plots are shown in green, and Rosy Hills plots are shown in orange.

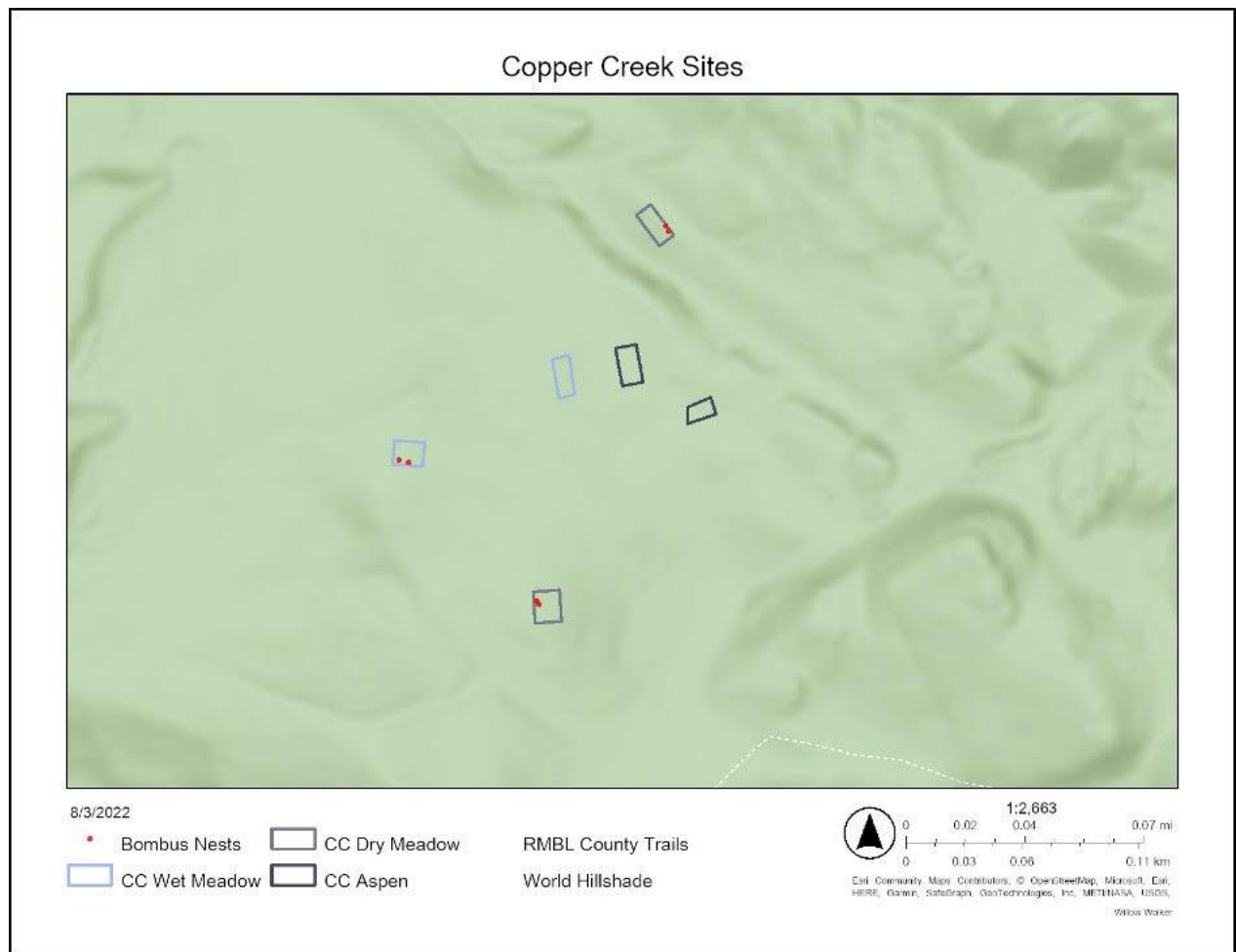


Fig. 2. This figure shows all the nest searching plots at the Copper Creek site. The lightest color represents plots in the wet meadow, the middle color represents plots in the dry meadow, and the darkest color represents plots in the aspen habitat. The red points show identified *Bombus* nests.

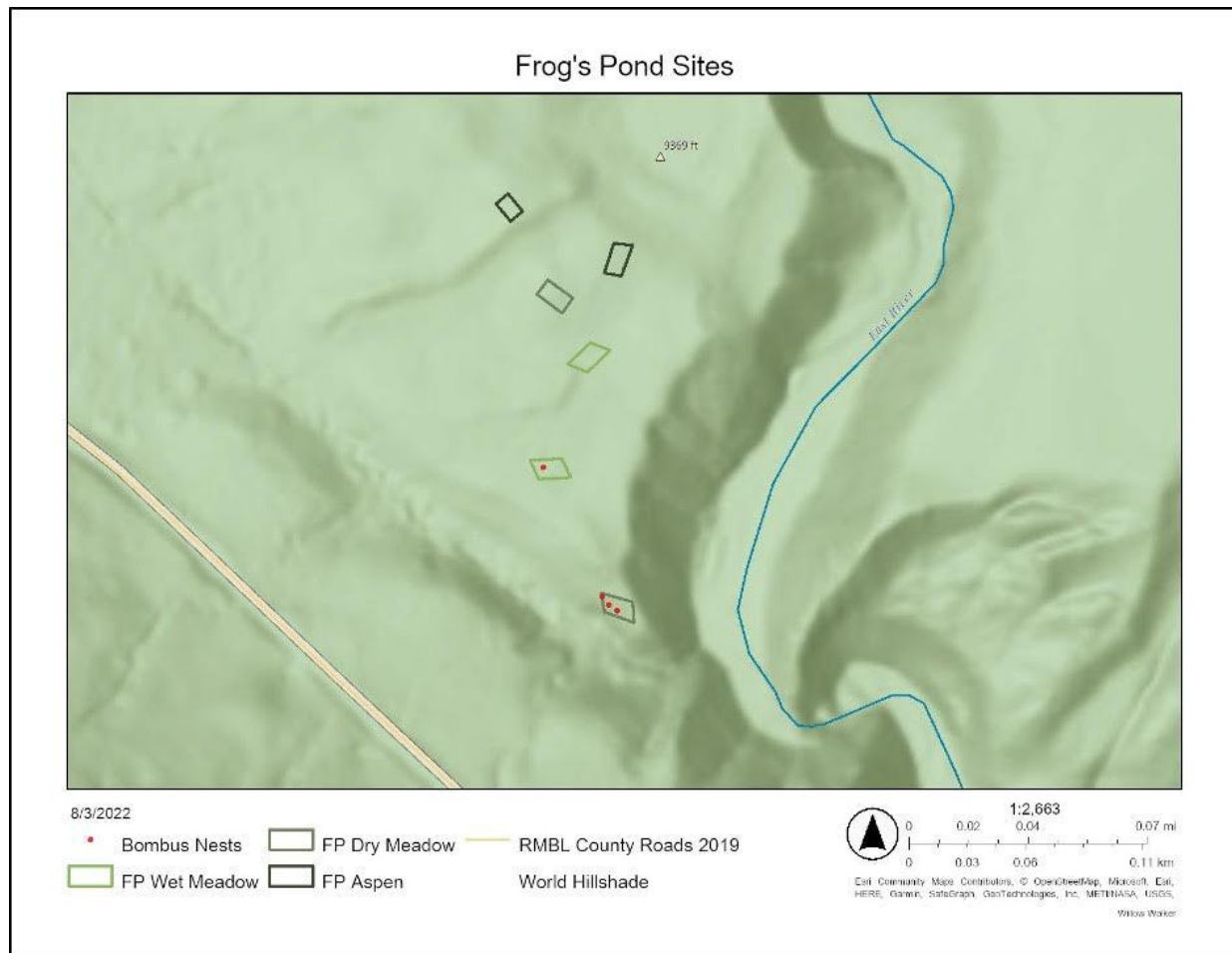


Fig. 3. This figure shows all the nest searching plots at the Frog's Pond site. The lightest color represents plots in the wet meadow, the middle color represents plots in the dry meadow, and the darkest color represents plots in the aspen habitat. The red points show identified *Bombus* nests.

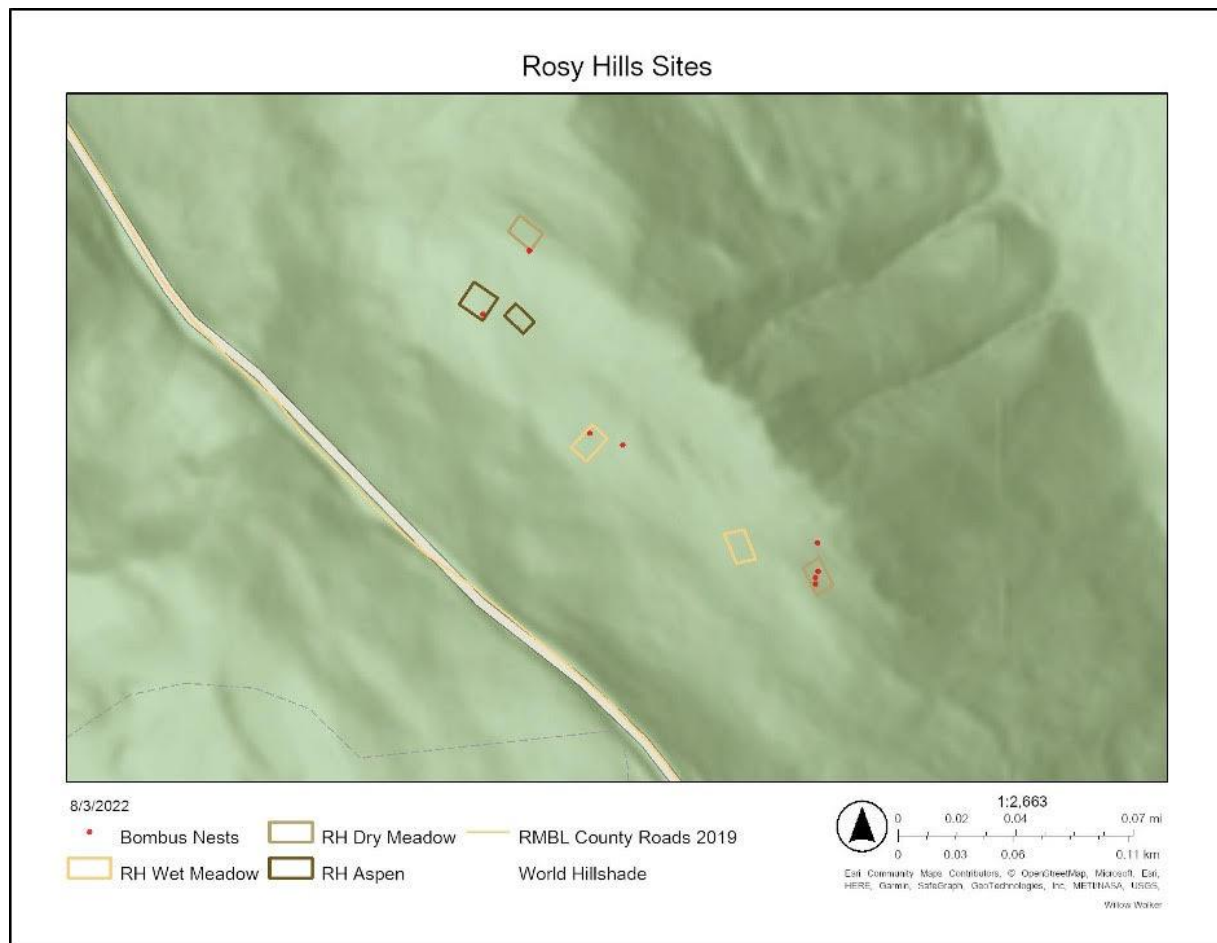


Fig. 4. This figure shows all the nest searching plots at the Rosy Hills site. The lightest color represents plots in the wet meadow, the middle color represents plots in the dry meadow, and the darkest color represents plots in the aspen habitat. The red points show identified *Bombus* nests.

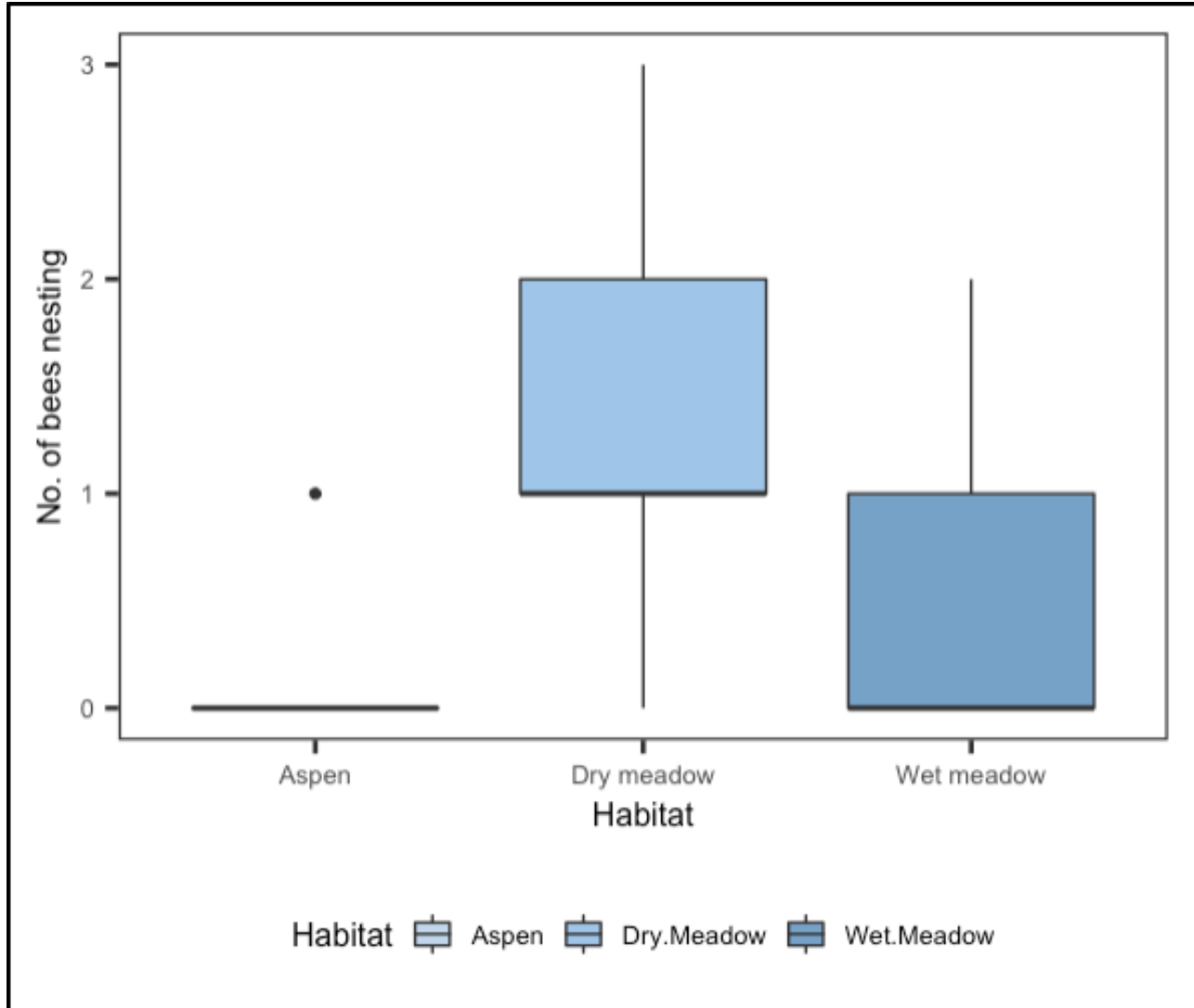
Graphs

Fig. 5. This figure shows the distribution of nests across different habitat types in box plot form. As described in the discussion section, there is significant difference between “Aspen” and “Dry Meadow” but no significant differences between “Wet Meadow” and either.

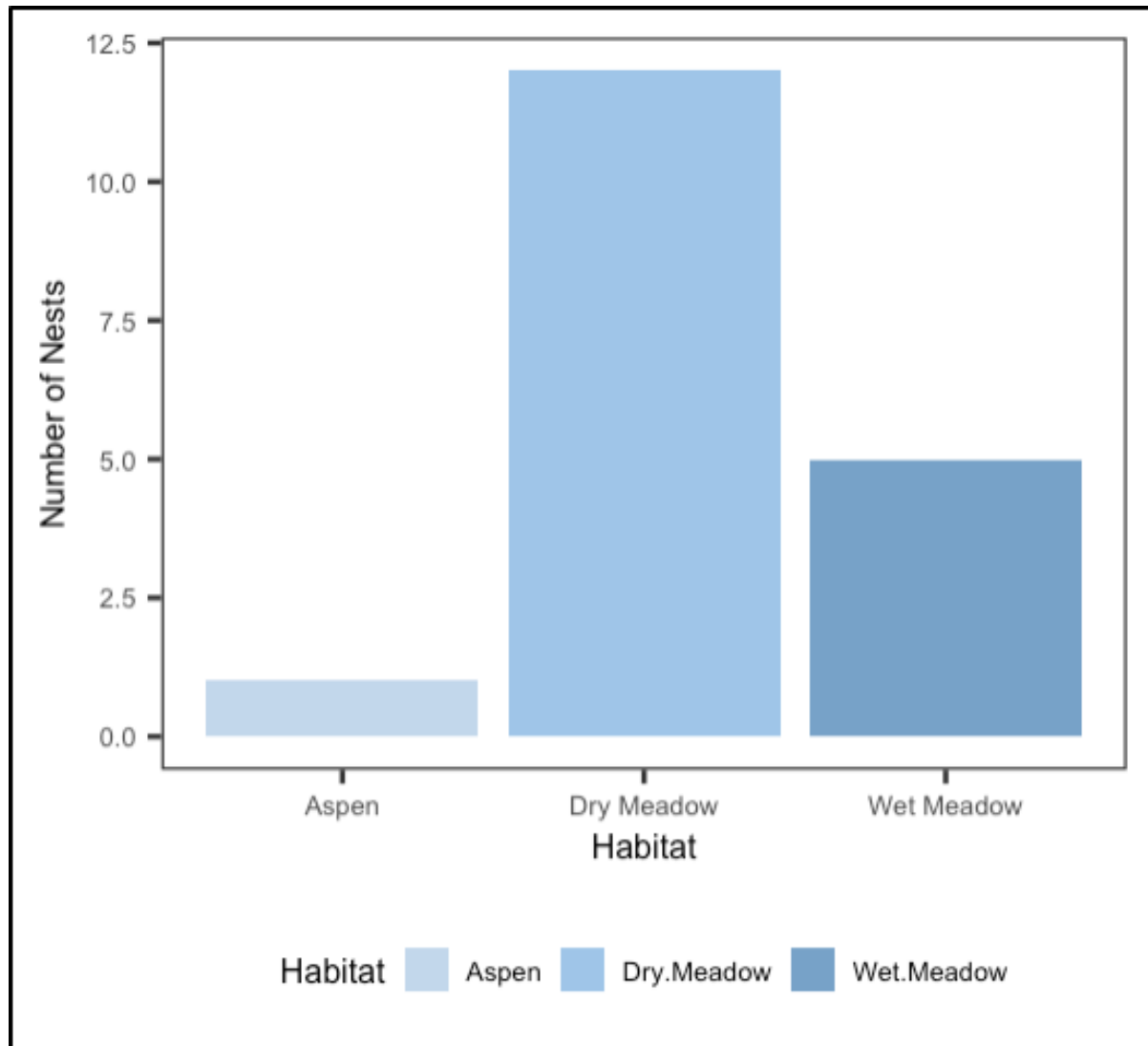


Fig. 6. This figure shows the distribution of the 18 *Bombus* nests identified. 12 nests were found in Dry Meadow, 5 in Wet Meadow, and 1 in Aspen.

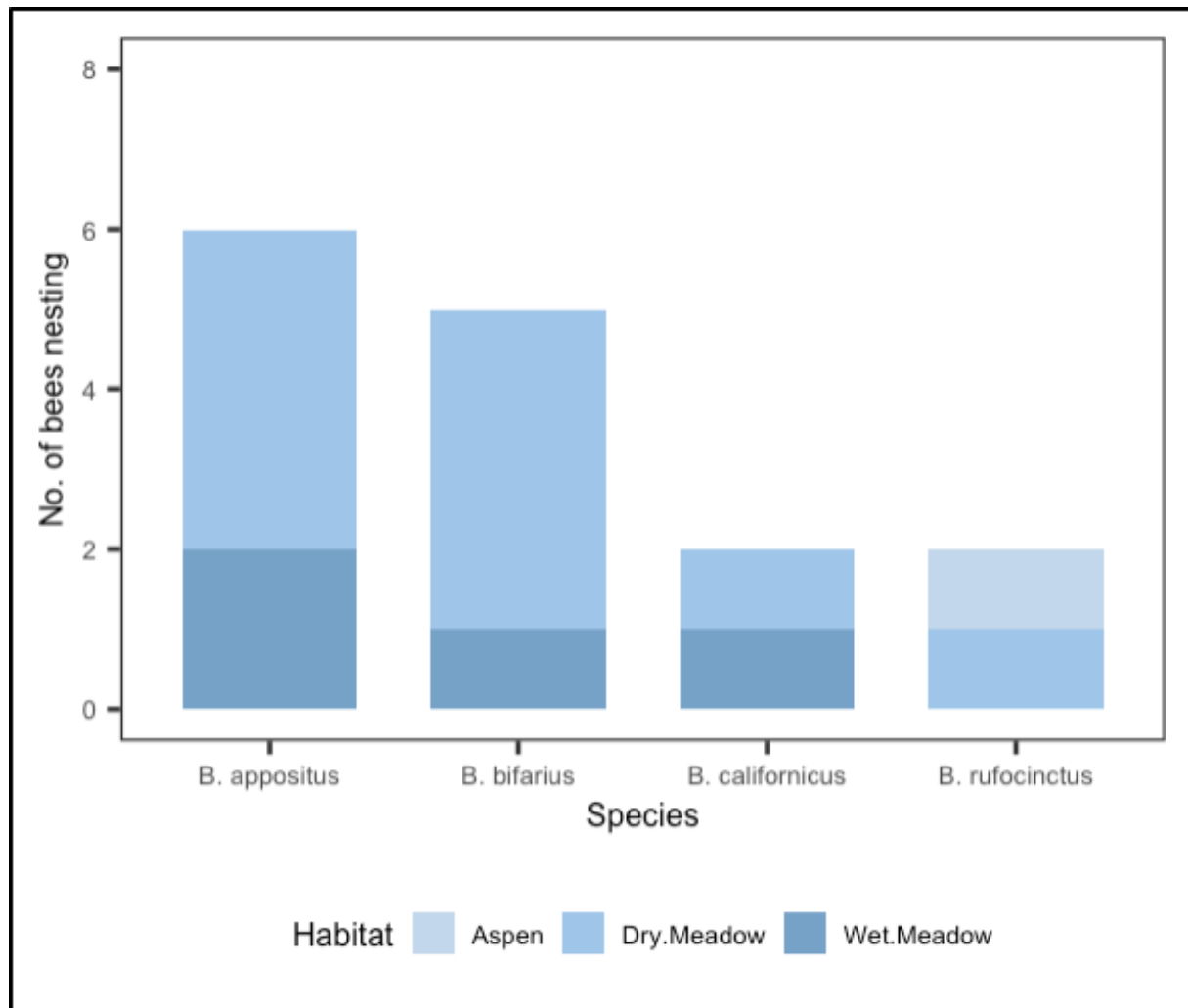


Fig. 7. This figure shows the distribution of nests across habitat types by species. As stated in the discussion section, although some *Bombus* species seemed to prefer a certain habitat, there was insufficient evidence to draw conclusions.

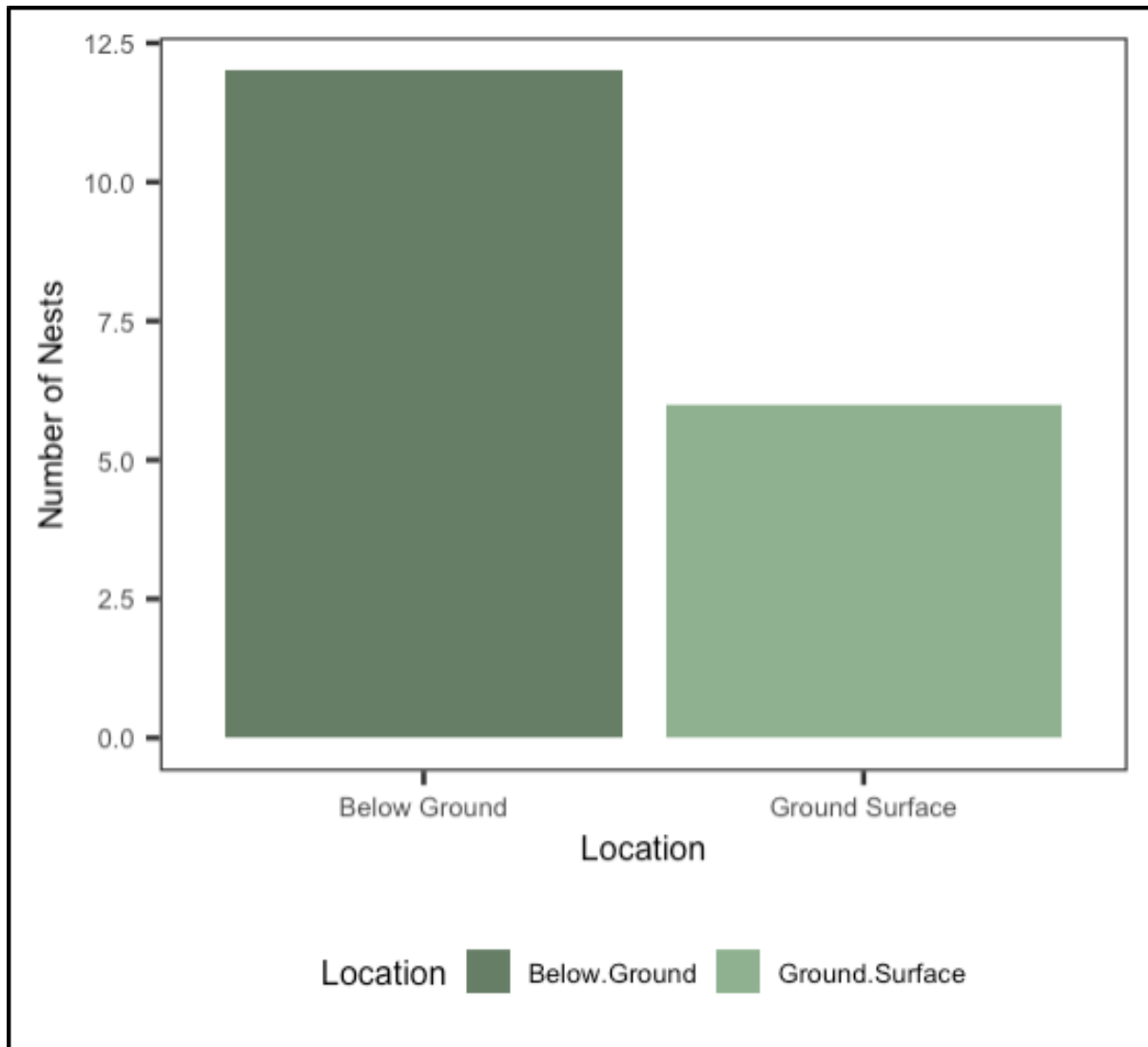


Fig. 8. This figure shows the distribution of *Bombus* nest location relative to the ground. As shown in this graph, 12 nests were found below ground while 6 were found on the ground surface. No nests were identified above ground.

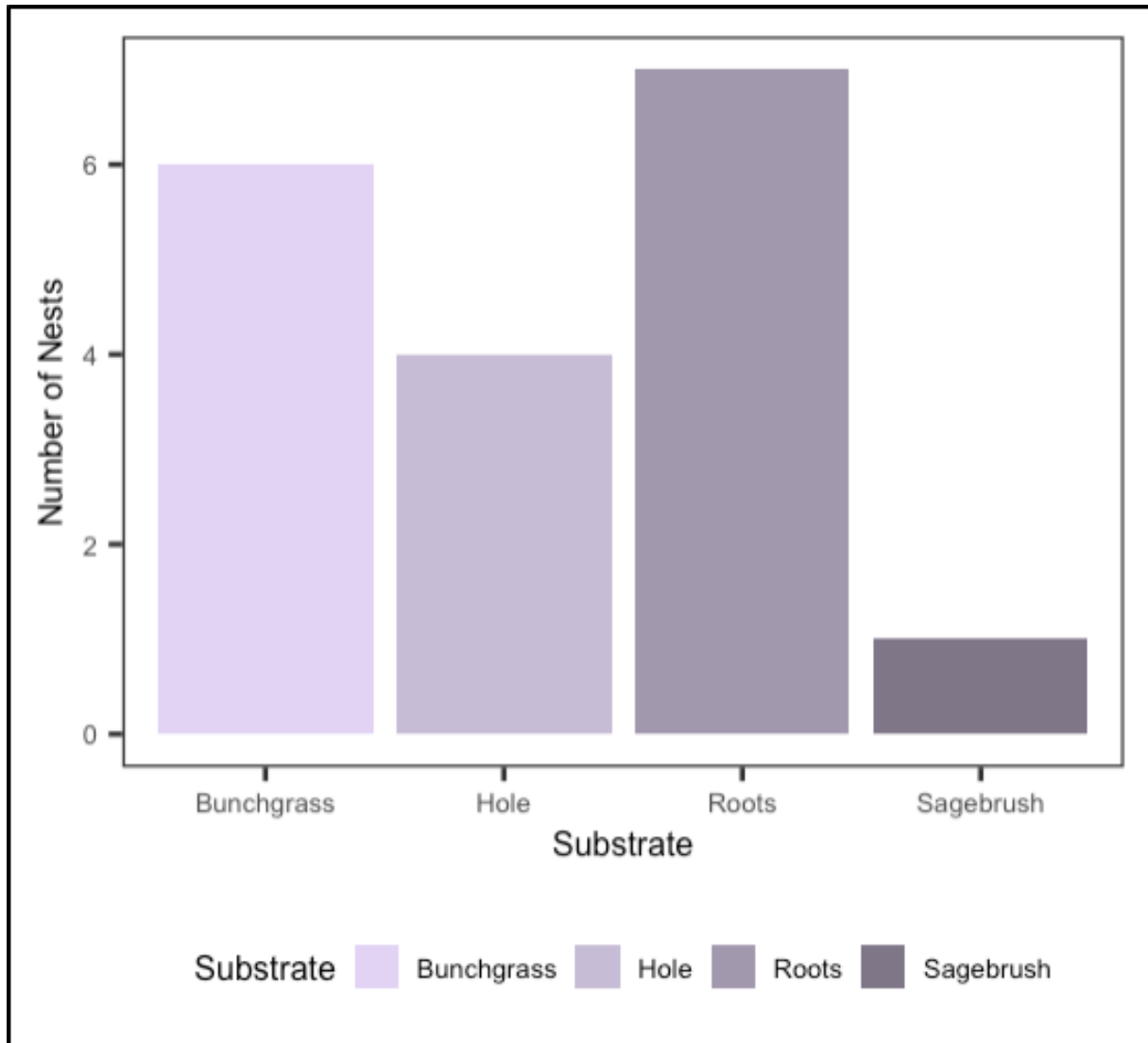


Fig. 9. This figure shows the distribution of nests based on substrate. As shown in the graph, 6 nests were found in bunchgrass, 4 in holes, 7 in roots, and 1 in sagebrush.