How does the presence of forbs and change in elevation affect the genus richness of butterfly populations at the National Bison Range?

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Abstract

Butterflies are important contributors to the ecosystem due to their many roles, which include serving as food source for many other species, functioning as pollinators and as indicators of environmental climate changes. Environmental factors do not only affect invertebrate communities, but vegetation as well, and in turn each other. It is expected that butterflies might act differently depending on the resource availability of the surrounding environment, and that the interaction between the butterflies and their landscape depends much on surface characteristics and elevation. The main purpose of this project was to determine if the richness of the butterflies changed as elevation and forb richness changed. Areas of high and low elevation were plotted, where butterfly and forb richness were determined. Our results indicate that only forb species richness correlates significantly with elevation, while butterfly richness does not. Furthermore, we found that butterfly and forb richness show a similar pattern only across the low-elevation sites. This finding can be better understood by setting up a project that includes more low-and high-elevation sites; further studies within this phenomenon are encouraged.

Keywords: butterfly genus richness, forb species richness, high elevation, low elevation

Introduction

Butterflies are important contributors to the ecosystem due to their many roles, which include serving as food source for many other species, functioning as pollinators and as indicators of environmental climate changes on the account that they are sensible to weather shifts. Butterflies are conspicuous components of open habitats and indicators of habitat quality (Kocher & Williams, 2000). Their limited dispersal ability, larval food plant specialization and close-reliance on the weather and climate make many butterfly species sensitive to fine-scale changes, emphasizing their potential role as indicators (Botham, 2006) of environmental welfare. Assessing their richness is valuable considering that an abundant butterfly community, might indicate balance and health in the environment. Although it would be expected to have a comparable species richness of butterflies each year where proper conditions for their development seem to be present, it is crucial to keep in mind that butterfly populations and optimal living conditions vary with species, and that populations living at the upper and lower altitudinal extremes experience quite different environmental conditions (Hodkinson, 2005). These changing conditions include temperature, precipitation, pressure, wind speed, and sun input. This has clear implications for our general understanding of the biology of butterfly species and their distribution (Hodkinson, 2005). Weather primarily affects individual butterflies by altering activity time and resource availability (Boggs & Murphy, 1997). This can either have a positive or negative effect for their species abundance and richness.

Environmental factors do not only affect invertebrate communities, but vegetation as well, and in turn, each other. For example, precipitation is a major factor affecting the diversity, distribution and productivity of insect host plants and the nature of the associated soils (Hodkinson, 2005). Various environmental conditions are in constant change depending on the altitude of the habitat. Changes on the natural conditions of low-elevation areas may lead to contrasting parameters of the biodiversity that is present. As a result, it is expected that butterflies might act differently depending on the resource availability of the surrounding environment, and that the interaction between the butterflies and their landscape depends much on space-time scales as well as surface characteristics (Dennis, 1993). This may mean that the dispersal of butterflies greatly varies with species size, food and plant specificity for future generations and that the local densities and regional distribution of species are not independent (Cowley *et al.,* 2001).

Within an assemblage, species that are locally common tend on average to be more widespread than those that are locally rare (Cowley *et al.,* 2001). Therefore, the tendency of observing the same species of butterflies in a determined area for a certain period of time is not uncommon. However, this does not mean that diversity cannot occur in a small perimeter. It all relates to the needs of each butterfly species, as well as every individual, and what the habitat provides. Spatial variability in species richness is a common phenomenon at different spatial scales and extents, and has formed the basis for innumerable ecological and evolutionary studies (Kerr *et al.,* 2001).

Climate is commonly observed to predict species richness better than topography-based measurements of habitat heterogeneity (Kerr *et al.,* 2001). Hence, since butterflies are among the invertebrates that are most sensible to climate changes, assessing their diversity and richness is key to knowing if they are still thriving, or if they are struggling for survival. Additionally, due to the importance of butterflies for environment health and wellness, a better understanding of their species' distribution, and what affects it, is crucial. This leads to asking: does the species richness of butterflies change with elevation and richness of forbs? Because of this, the main focus of this project is to assess the richness and diversity of the butterflies, as well as for the forbs that are present throughout the study area, to examine how butterfly richness changes across different elevations and forb compositions. I hypothesize that species richness of butterflies will increase as forb richness and elevation increase. This is due to high elevations resulting in high resource availability, where the general tendency is for UV radiation input to rise with increasing altitude (Hodkinson, 2005).

Elevation plays an important role for making enormous differences in radiation receipt, which influence temperature, humidity and other attributes (Dennis, 1993), such as precipitation which often rises significantly along an altitudinal gradient (Hodkinson, 2005) and results in higher soil moisture, creating an ideal environment for vegetation growth. This would mean that the probability of more diversity high on the mountains is greater, providing a boost in diversity.

Methodology

Study site

This research study was conducted at the National Bison Range in western Montana. This wildlife refuge is administered by the U.S. Fish and Wildlife Service, and consists of 18,800 acres of intermountain habitat, along with Palouse and Bunchgrass prairie. This project was conducted at 4 different locations within the limits of the NBR. The locations included three low-elevation sites, similar to a prairie, and three high-elevation sites, with more woody vegetation and rocky soils (see Table1).

Setting the plots and forb identification

For each category, 6 sites were chosen, each with a 20x20 m^2 plot. In the middle of each said plot, another smaller 10x10 m^2 plot was measured. Next, one horizontal transect was set along the outer line of each of the smaller plots, marking with flags every 2 m. Then, at each of these flags, a vertical transect was set, again marking every 2 m. Finally, at each of the vertical transect flags, a 1 m diameter circle was measured (see Figure 5 for a visual representation of the plot).

The forbs were counted and identified within each of the 1 m diameter circles, having a total number of 16 circles inside each plot. All live forbs were counted and identified, including the juveniles, independent of their blooming state. All dead or dry individuals were not counted. This process was done once a week, for two weeks.

Butterfly sampling and identification

Each of the sites were sampled for butterflies a total of six times, this being done from one to five days immediately after forb sampling was carried out. Each sampling consisted of 20 minutes of active counting and trapping of the observed individuals. Within the 20 minutes, all butterflies that stopped to forage or that flew over the plot were counted, and all counted butterflies were attempted to be captured using butterfly nets. At the end of all butterfly sampling, we had captured 32 % of all observed individuals. The butterflies that could not be captured were identified by sight. If the uncaptured butterfly could not be distinguished between a similar genus, it was recorded as the more common of the genera being referred to in the area. The captured butterflies were placed in kill jars with 1-2 cotton balls drenched in acetone and each site had corresponding separate jar. The collecting process happened any one day between 10:00 and 17:00 for a period of 2 weeks during the month of July 2018 (see specific dates in Table 1). Sampling did not happen in rainy or windy days. Additional data taken includes daily temperature, site elevation and coordinates.

At the end of each sampling day, all captured butterflies were taken to the laboratory, where they were counted and identified to genus. That was made to decrease the chances of misidentifying species that belong in the same genus. In order to have visual references for identification of future captures, at least one individual butterfly of each genus was pin-mounted. The resulting total number of genera was eight: Cercyonis, Colias, Hesperia, Lycaeides, Papilio, Phyciodes, Pieris and Pontia.

Statistics

All statistical analyses were done using R (R 3.5.1) and RStudio (1.1.456). First, two individual one-way analysis of variance (ANOVA) tests were done to evaluate the relationship between the genus richness of butterflies vs. the site elevation, and forb species richness vs. site elevation. Second, a Tukey post-hoc test was done to verify if there was a significant difference across the distinct sites. Lastly, a Welch t-test was done to see if there was a significant relationship between butterfly genus richness and elevation. The same test was made for the species richness of forbs.

Results

The first ANOVA test indicates that butterfly genus richness is significantly different across sites (Table 2, f-value = 3.779, p-value = 0.00898). This was also true for forb species richness across sites (Table 2, f-value = 9.829, p-value = 0.00744). After these tests we ran a Tukey post-hoc test in order to determine which sites pairs of sites had a significant difference between them of butterfly or forb richness (see Table 3). Results showed that four pairs of sites had a significant difference between them for forb species richness, but only one pair of sites had a significant difference in the case of butterfly genus richness (Table 3, p-values < 0.05).

In order to assess whether there is a relation between butterfly genus richness and elevation of sites, we did a Welch t-test, however, there no significant relationship was found (Table 4, t = -1.5177, df = 3.3709, p-value = 0.1384). On the other hand, the results showed that forb species richness does have a positive relation with site elevation (Table 4, t = 5.4584, df = 7.5002, p-value = 0.007509). Additionally, there was no significant relationship between butterfly genus richness and forb species (Table 4, t = 4.7548, df = 3.6803, p-value = 0.2463).

Discussion

This study was carried out to test the relationships between butterfly richness with forb richness and elevation. Although the results do not fully support my hypothesis that butterfly richness would increase, it was shown that forb richness did increase with elevation. The results are a giveaway of how variant and dynamic butterfly populations are.

In order to better understand how butterfly conservation can be achieved, it is substantial to study their population dynamics (Hawkins *et al.,* 1997), understanding first what population dynamics means. Population dynamics is the study of how and why population numbers change with time and space and document the empirical patterns of population change, while attempting to determine the mechanisms that explain the observed patterns (Turchin, 2003). For butterflies, the dynamics that characterize each species is subjected to the habitat they live in, which affect their survival and adaptation to the utilization of the available resources. Dynamic environments can change constantly and suddenly, therefore making it hard to determine what limiting factors individuals may encounter at the beginning of their lives. Despite this, there is still a diversity of butterfly communities that survive these habitats, thus making it more interesting to determine how different environments mold their presence.

The National Bison Range topography differs at many places within the limits of the property. There are many areas that vary in elevation, ranging from less than 800 m to almost 1,500m above sea level. These contrasting landscapes suggest that elevation can have an effect on butterfly richness, primarily because changes in elevation can sometimes imply more soil moisture, more vegetation, different sun impact, etc. However, this was not the case.

The results of the ANOVA test, indicated that there is an existing, significant difference between distinct sites and richness of butterflies and forbs. This means that the specific nature of each site did impact butterfly and forb richness. It was shown through the Tukey post-hoc test that, or the butterflies, only two out of six sites had a significant difference between them when calculating genus richness; these sites were Tower 1 (B) and Northgate Road. In this case, Tower 1 is a high-elevation site, but resulted in the least richness of butterflies among all sites, whereas Northgate Road, a low-elevation site, ended conceiving the highest butterfly genus richness. The situation was somewhat different for the forbs, having four pairs of sites that were statistically different between each other. These resulting pairs were the sites with the highest elevations, like

Tower 1 and Tower 2, against the sites with the lowest elevations (Table 1). The results showed that in this case, forb richness was highly impacted by the locations, more so than butterflies.

Moreover, in order to test if there was a direct relation between elevation and the mean richness of butterflies and forbs, we did a Welsh t-test. The results of this test suggest that forb species richness does increase along with elevation. Those effects could be a single variable, like soil moisture, or a combination of other such as moisture, sun radiation, slope, etc. Despite the correlation between forb species richness and elevation, the same could not be said about butterfly richness and elevation. Our hypothesis stated that butterfly communities would have higher richness in places were the main food resource (forbs, for example) was abundant, but the results state otherwise. It can be concluded that in this study butterfly richness did not correlate with the presence of forbs.

Furthermore, the last test made was a StepAIC, which indicated that the model was not strong enough to predict significant results. Some explanations about why we got several non-correlations are that the number of replicates could be too low for the tests to detect a pattern. Additionally, perhaps the number of butterflies counted was still not enough to assess their richness as accurately as possible. Other environmental factors, like wind, heat, disturbances, etc., might have had an impact on the butterfly behavior, thus impacting my results.

In conclusion, the hypothesis for this study was not fully supported, since elevation is not a factor that significantly affects butterfly richness. What is it then, if not elevation, what makes butterfly genus richness change? Previous studies made by Kerr et al. 2001 tried to predict butterfly richness and established that it is not just one factor, but a combination of settings that are used to try to predict their richness. Examples of those settings are the latitudinal position, the climate, spatial variability and topography. Ultimately, trying to predict a certain pattern or behavior in a changing planet is always a challenge, considering how many factors should be taken into account and how climate can shift across years.

The population dynamics of butterflies is in constant change, subject to every species' need and to many environmental factors that are also in constant change. Nonetheless, studying butterflies will help scientists assess environment health and develop better conservation strategies. Lastly, for future studies of species richness, I recommend taking into account certain helpful variables, such as peak seasons of different butterfly species, and increasing the amount of sampling days and crew members. Also, since our results indicated that butterfly and forb richness show a similar pattern only across the low-elevation sites. For this, we would recommend having a higher amount of low-and high-elevation sites ran against butterfly richness so as to be further elucidate this in a future study.

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Tables and Figures

Table 1. Study sites information.

Location	Dates sampled (July 2018)	Coordinates	Elevation (m)
Pauline (A)	11, 12, 13, 18, 19, 20	47.3458544, -114.2762976	795
Pauline (B)	12, 13, 18, 19, 20, 21	47.3429008, -114.2762269	795
Northgate Road	11, 12, 18, 19, 21, 22	47.3688637, -114.2114572	850
Tower 1 (A)	11, 12, 13, 18, 19, 21	47.3445088, -144.2316254	1240
Tower 1 (B)	11, 12, 13, 19, 21, 22	47.3415129, -144.2278603	1130
Tower 2	11, 12, 13, 18, 19, 20	47.3175669, -144.2444829	1370

Table 2. Butterfly and forb richness across sites. The given values suggest that there is a relation between the sites and butterfly genus richness and sites and forb species richness.

Relation between butterfly genus richness and sites	
f-value	3.779
p-value	0.00898

Relation between forb species richness and sites	
f-value	9.829
p-value	0.00744

Table 3. Difference between study sites and richness of forbs and butterflies. The p-values found on this table mean that there were certain sites that had a major difference in term of the richness of forbs and butterflies found there. We can see that the butterfly richness is more variant only between two site, but it

does not differ much between other sites, unlike the great variety of richness difference amongst forbs and sites.

Significant difference between site richness for forbs and butterflies	
Site difference for butterfly genus richness	p-values
Tower 1 (B) - Northgate road	0.0030598
Site difference for forb species richness	
Tower 2 - Pauline (A)	0.0374983
Tower 1 (A) - Pauline (B)	0.0195429
Tower 1 (B) - Pauline (B)	0.0269144
Tower 2 - Pauline (B)	0.0106660

Table 4. Testing relationship between butterflies, forbs and elevations. We used a Welsh t-test to test the relationship between all variables. Forb species richness vs. elevation was the only statistically significant relation. Butterfly genus richness did not have a significant relation with either elevation nor forb species richness.

Butterfly genus richness vs. Elevation	
t	-1.5177
df	3.3709
p-value	0.1384
Forb species richness vs. Elevation	
t	5.4584
df	7.5002
p-value	0.0007509
Butterfly genus richness vs. Forb species richness	
t	4.7548
df	3.6803

p-value	0.2463

Table 5. Testing relation between forb richness, butterfly richness and elevation. The results of this linear model shows no relation between richness of forbs, richness of butterflies and elevation.

	estimate	st. error	t-value	p-value
(Intercept)	21.79087	11.77607	1.850	0.161
Elevation	-0.02228	0.03055	-0.729	0.519
Mean richness of forbs	0.70553	1.12772	0.626	0.576

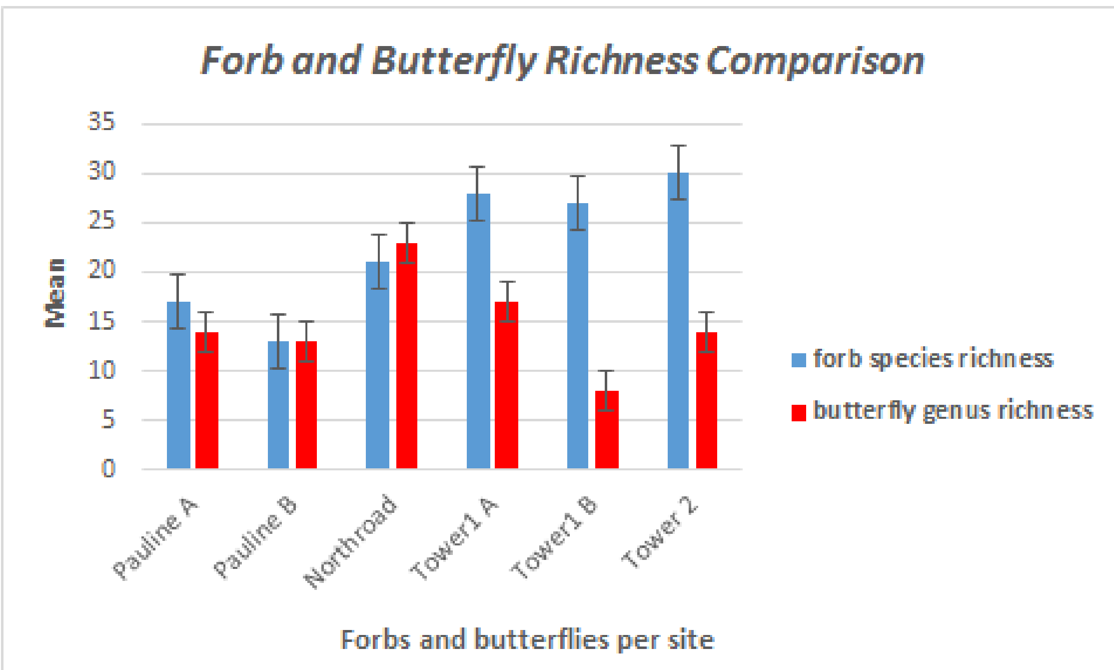

Figure 1. Means of richness comparison for each site. The comparison between the average of the genus richness of butterflies shows that there is an apparent correlation between the butterfly and forb richness, but only at the lower elevation sites.

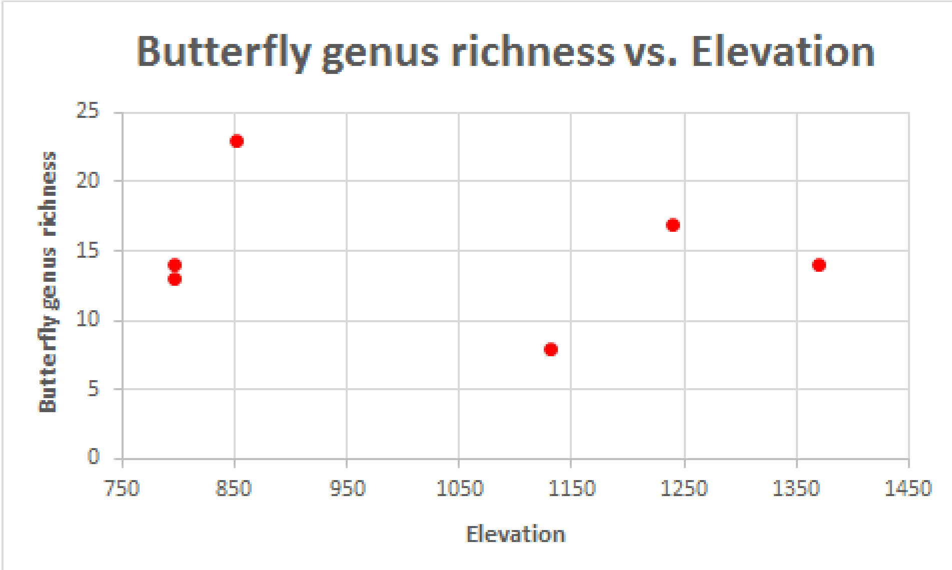

Figure 2. Comparing butterfly genus richness and elevation. It can be noted that butterfly genus richness and elevation does not seem to have a significant correlation (t = -1.5177, df = 3.3709, p-value = 0.1384).

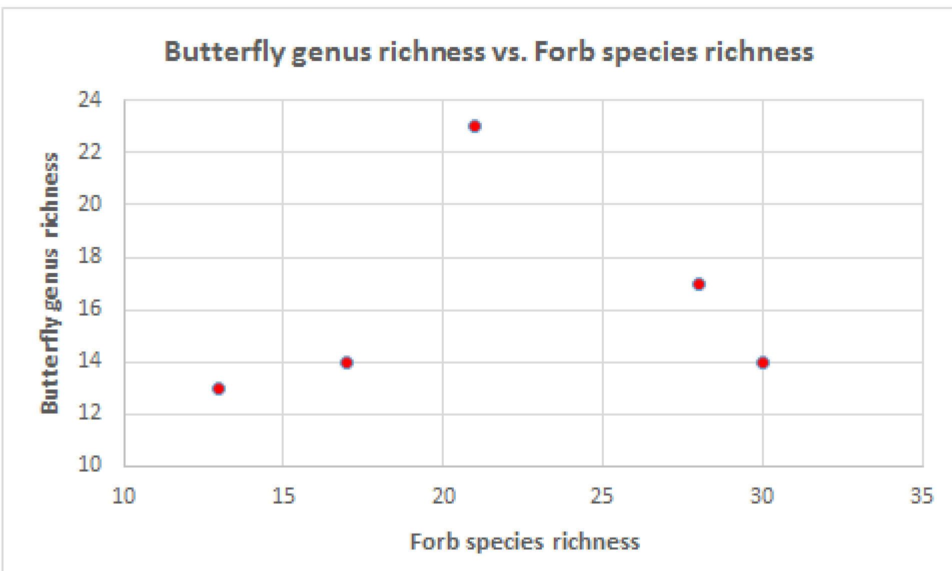

Figure 3. Comparison between butterfly genus richness and forb species richness. This scatter plot shows that butterfly genus richness does not have a significant relation with forb species richness (t = 3.7548, df = 3.6803, p-value = 0.2463).

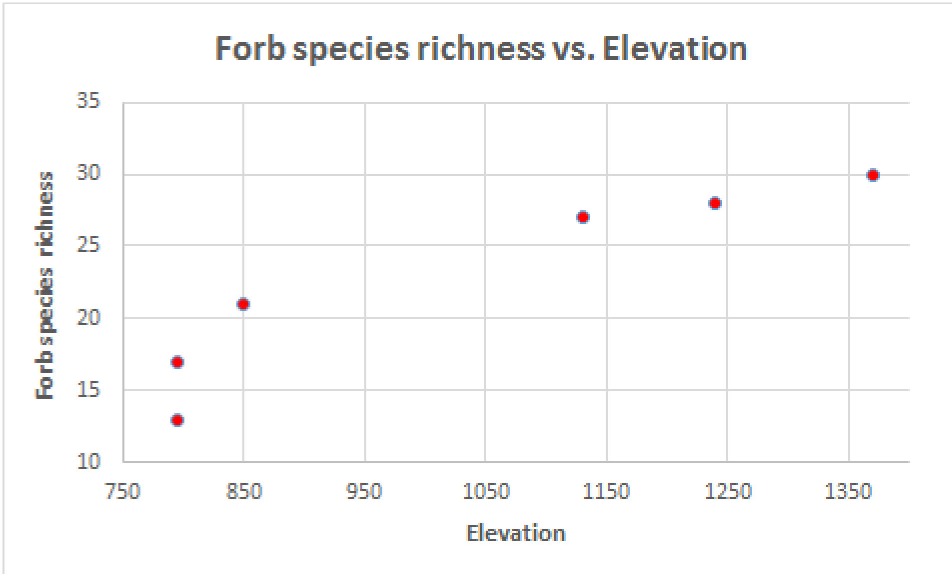

Figure 4. Comparison between forb species richness and elevation. This scatter plot shows an increase of forb species richness as elevation increases ($t = 5.4584$, $df = 7.5002$, p-value = 0.0007509).

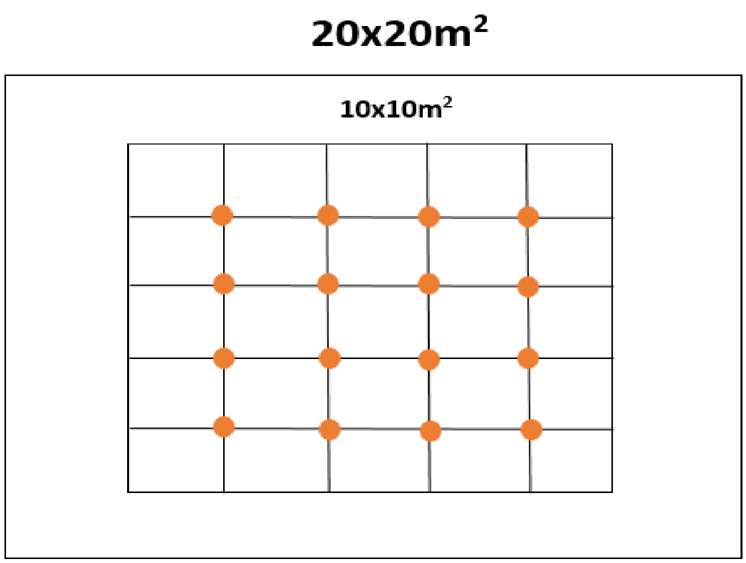

Figure 5. Plot diagram. This figure shows how the sampling plot was set. The colored dots represent the areas where the forbs were sampled (within a 1 m diameter circle at each orange dot). Butterflies were counted the moment they flew over the 20x20 m^2 square.