The Relationship between *Trifolium* spp. Abundance and Environmental Variables in East Bay Grasslands

Amber Dalke

Abstract California grasslands are complex, unique ecosystems comprised of many species of native and nonnative vegetation. One group of species widely used for range improvement is the *Trifolium* (wild clover) genus. However, *Trifolium* are among the more understudied vegetation types. *Trifoliums* are important to grassland ecosystems because they replenish deficient nitrogen in N-limited ecosystems and serve as forage when food is scarce. Therefore, understanding clover abundance in relation with environmental variables is important in the management of California's vulnerable grasslands. To examine these relationships an observational study was conducted in 63 plots within seven parks of the East Bay Regional Park District over three years (2005-2007). Variables tested relating to *Trifolium* abundance included geographical variables (slope, aspect, elevation, and heat load) and soil composition (total N, total C, P, X-Ca, sand, silt, clay, and pH). Of the *Trifolium* found, the majority came from a single nonnative species, *T. hirtum*. With the use of linear regressions, this study found *Trifolium* spp. abundance positively related to slope ($\bar{x}R^2=0.22$, $\bar{x}p=0.01$) and elevation ($\bar{x}R^2=0.25$, $\bar{x}p=0.02$) throughout the three-year time frame. Other relationships were observed including a positive relationship to silt ($R^2=0.20$, $p=<0.01$) in 2005, a negative relationship to pH ($R^2=0.10$, $p=0.05$) in 2005, and negative relationships to total N ($\bar{x}R^2=0.15$, $\bar{x}p=0.04$) and total C ($\bar{x}R^2=0.57$, $\bar{x}p=0.06$) in 2006 and 2007. This research provides an important description of variables that may influence the abundance and distribution of *Trifolium* spp. in the East Bay.

Introduction

Of the many environments found in California, one of the more ecologically important ecosystems is valley grasslands (Biswell 1956). California valley grasslands are located in the treeless belt that borders the cultivated regions of the Central Valley (Holland 1995), consisting of 5.35 million hectares (Kuchler 1964). The climate of the region is Mediterranean, which is characterized by cool, wet winters and hot, dry summers (Holland 1995). Plants introduced from similar environments with separate evolutionary histories have successfully adapted and mixed with California's native vegetation (Baker 1989). Due to this mixing, California grasslands house unusual plant communities consisting largely of nonnative, annual, naturalized grasses and forbs (Holland 1995). Of the many species found in the valley grasslands of California, *Trifolium* spp. (wild clover) is among the more favorable vegetation types in terms of their contributions to soil nutrition (Green 1959) and their ability to produce forage for grazing animals through the majority of the year (Murphy *et al.* 1973). Therefore, determining the environmental variables under which *Trifolium* is most abundant will aid in the management of California grasslands.

Trifolium spp., both native and nonnative, are ecologically important to rangeland ecosystems in terms of their effect on soil composition. Soils of California grasslands can be deficient in nitrogen (N) (Jones and Woodmansee 1979). As a member of the Fabaceae family, *Trifolium* spp. may improve N availability (Weitkamp and Graves 1987). This is accomplished through nodules on the root of clovers (Weitkamp and Graves 1987). The nodules are formed by the invasion of a bacterium, *Rhizobium*, which has the ability to fix gaseous N in simple and complex organic and inorganic compounds (Green 1959). This allows more productive grasslands through the replenishment of depleted soil N (Jones and Woodmansee 1979).

Trifoliums are also ecologically important due to their ability to produce viable "forage" throughout the year. *Trifolium* spp. has been used to increase livestock production by improving the quality and availability of winter, spring, and early summer forage (Graves *et al.* 2001). This can be mainly attributed to the fact that the foliage, both green and dry, is good feed for livestock and wildlife (Murphy *et al.* 1973). One species, *T. repens* (white clover), is widely regarded as a highly nutritious rangeland plant (Ulyatt 1980). This is due to the high protein content and low structural fiber of the forb allowing for easier intake and digestion by grazers (Ulyatt 1980).

Past research on the effect of environmental variables on *Trifolium* spp. abundance in California has been limited to studies dealing with the effects of N and phosphorus (P) addition.

Of the studies dealing with N, findings have shown that the percentage of clover abundance was decreased where N was applied through fertilizers (Jones 1966; Jones and Winans 1967). In terms of P, Bentley (1946) found that legumes, particularly clovers, are more abundant on soils containing more P. Clovers growing without sufficient P are short with small, dark-green leaves as compared to clovers growing with adequate amounts of P (Murphy *et al.* 1973). Similar to past findings, Jones and Evans (1960) found that *Trifolium* spp. abundance increased with the addition of P. Past research is limited to analysis of N and P additions and should be expanded to include geographical variables and other soil components.

Of the many species of *Trifolium*, there are two nonnative species widely distributed in California. The first is *T. hirtum* (rose clover). Rose clover, a native to the Mediterranean region and Asia Minor (Love 1985), was first planted in 1944 in Sacramento County (Murphy *et al.* 1973). Rose clover is a versatile, annual forb and it is able to live on harsh environments of rocky, poor soils (Murphy *et al.* 1973). Rose clover is also an excellent forerunner to other clovers on low fertility soils and aids in the overall improvement of rangelands (Murphy *et al.* 1973).

The other common nonnative species of *Trifolium* is *T. subterraneum* (subclover). It is native to the Mediterranean area, but was naturalized and brought from Australia, arriving in California in 1933 (Murphy *et al.* 1973; Graves *et al.* 2001). Subclover is an annual forb with more temperamental preferences than rose clover. It requires neutral to moderate soil acidity and elevations below 120 m (Murphy *et al.* 1973). Like rose clover, subclover can aid in the improvement of rangelands (Murphy *et al.* 1973).

Currently there is little research done specifically on *Trifolium* spp. (Lulow 2007). However, one of the major challenges to the restoration of diversity is the management of native forbs, including *Trifolium* spp. (Lulow 2007). *Trifolium* spp. represents a significant and highly diverse forb group in California grasslands (Lulow 2007). However, the clover family remains understudied despite the fact that nonnative clovers have been used extensively for range improvement (Lulow 2007). Without proper management and an absence of *Trifolium* spp., it is possible that grasslands will be composed of less-nutritious forage (Lulow 2007). However, it is unclear whether other legumes would be present if *Trifolium* abundance were decreased. For this reason, a thorough understanding of clover abundance in comparison with environmental variables is needed in order to better manage this forage species.

This study will present important information for the purpose of understanding *Trifolium* spp. in relation to environmental variables. This study also has possible implementation for habitat management in terms of restoring sites to expand the number of both legumes and forbs in rangeland ecosystems. This research will bring much needed attention to conservation, restoration, and management of the clover community.

East Bay grasslands, specifically the grassland ecosystems within the East Bay Regional Park District (EBRPD), are ideal to study *Trifolium* spp. in comparison with environmental variables. The sites consist of healthy populations of both native and nonnative grasses and forbs distributed over a wide range of environmental factors. This study asks, "What is the relationship between *Trifolium* spp. abundance and environmental variables in East Bay grasslands?" Geographical factors (slope, aspect, elevation, and heat load), soil composition (total N, total C, X-Ca, P, sand, clay, silt and pH), and *Trifolium* spp. abundance were measured to determine if correlations were present. Based on previous research, I hypothesize that there will be a greater *Trifolium* abundance on soils that contain less total N and higher content of P. Furthermore, I hypothesize that the other environmental variables considered in this study will have no relationship to *Trifolium* spp. abundance.

Methods

In order to determine *Trifolium* spp. abundance in East Bay grasslands, vegetation surveys were conducted in the valley grassland subtype of the EBRPD. This included Brushy Peak Regional Preserve (Livermore, CA), Lake Chabot Regional Park (Castro Valley, CA), Morgan Territory (Livermore, CA), Pleasant Ridge (Pleasanton, CA), Sunol Regional Wilderness (Sunol, CA), Sycamore Valley (Danville, CA), and Vasco Regional (Brentwood, CA). These sites were chosen because they are representative of East Bay grassland ecosystems and consist of both native and nonnative grasses and forbs. The U.C. Berkeley Range Ecology Lab had previously established 63 randomly selected permanent plots. The broader purpose of this continuing study (2003-2013) is to develop and apply quantitative monitoring methods in order to determine the response of grassland communities to rangeland management. The permanent points were first located using a Global Positioning System and marked using a 1m piece of rebar inserted into the plots center, each with a radius of 17m. Vegetation data used in this study were collected between 2005 and 2007, with each plot surveyed once after the peak of the growing season between April and June.

The vegetation surveys were conducted using the point intercept method along four transects within each plot. Using a compass from the center of the circular plot, a 17m measuring tape (transect) was laid in the four cardinal directions. A 1m metal rod was vertically suspended along the transect and according to the larger study's protocol, the first species that the rod intercepted was recorded as the "hit". This method provided a systematic-random, yet consistent, system of sampling within each plot and ensured that the same transect will be sampled each year. For the first 4.5m of the transect, hits were recorded every 10cm. For the remaining 12.5m, hits were recorded every 0.5m. The change in scaling allowed many data points to be obtained and observations of small-scale and large-scale variations in plant species to be recorded. Overall, each of the 63 plots consisted of 280 hits. The percentage of the total number of *Trifolium* hits over the total number of hits was used as an index of cover. The species that were not identified by sight or with the help of the University Jepson Manual were collected and identified in the lab or by the staff of the Jepson Herbarium.

The Range Ecology Lab collected data on the geographical and environmental variables of each plot once in 2006. Geographical factors (elevation (m), slope (%), aspect (degrees), and heat load ($(1\text{-cosine}(\text{aspect}-45^{o}))/2)$) were determined for the center of each plot using digital mapped data and a Geographic Information System. Heat load is a measurement of the orientation to the sun indexed from zero (northeast, coolest slope) to one (southwest, warmest slope) (McCune and Grace 2002). To analyze soil composition, four soil cores (length=10cm, radius=5cm) were taken from each plot at the 8m mark along each transect. This point was selected because it is roughly in the middle of the plot and represents an average composition for the entire transect. The cores were blended into a homogenous mixture and sent to the Division of Agriculture & Natural Resources Lab (Davis, CA) for composition analysis. Total nitrogen (%), total carbon (%), phosphorus (ppm), exchangeable calcium (meq/100g), sand (%), clay (%), silt (%), and pH were measured.

The data were compiled and entered for all plots and years. Any species not identified as a *Trifolium* spp. was removed from the data set. A linear regression was performed with *Trifolium* spp. abundance and each of the environmental variables identified. This method was a useful tool for looking at individual variables. Any relationship with a p-value of 0.1 or less was considered to be significant. Thus, there was a ten percent chance of a false positive or rejection of the null hypothesis when it was true. However, the ability to find more and possibly weaker relationships was considered more important than the increased chance of a Type I error.

Results

Fifteen species of *Trifolium* were identified in the study. The most common nonnative species encountered was *T. hirtum* accounting for 72% of the overall *Trifolium* hits. Natives were comparatively much lower in abundance, representing 14% of all *Trifolium* hits, with the most common species being *T. bifidum* (notchleaf clover), *T. gracilentum* (pinpoint clover), and *T. willdenovii* (tomcat clover). Additionally, 2% of *Trifolium* hits could not be identified and are considered unknowns in this study. Other species identified included *T. barbigerum* (bearded clover), *T. campestre* (hop clover), *T. ciliolatum* (tree clover), *T. dubium* (shamrock), *T. glomeratum* (clustered clover), *T. macraei* (chilean clover), *T. microcephalum* (small-headed clover), *T. microdon* (Valparaiso clover), *T. oliganthum* (few-flowered clover), *T.* subterraneum, and T.*wormskioldii* (cow clover). Overall abundance varied over the three year time period (2005: 6%, 2006: 2%, and 2007: 3%).

Table 1: Results of linear regressions between environmental variables (Env. Var.) and *Trifolium* spp. abundance (cumulative abundance of all *Trifolium* spp., separated by year) based on measurements made between April and June of 2005, 2006, and 2007.

	2005		2006		2007	
Env. Var.	R^2	P-value	R^2	P-Value	R^2	P-Value
Aspect	0.054	0.137	0.085	0.112	0.013	0.508
Heatload	<0.001	0.895	<0.001	0.995	<0.001	0.943
Slope	0.247	**0.001**	0.228	**0.007**	0.189	**0.007**
Elevation	0.434	**<0.001**	0.191	**0.014**	0.124	**0.032**
N Total	0.017	0.414	0.110	**0.068**	0.182	**0.008**
C Total	0.039	0.211	0.098	**0.087**	0.155	**0.015**
P	0.063	0.109	0.058	0.190	0.065	0.128
X-Ca	0.049	0.158	0.011	0.579	0.007	0.630
Sand	0.014	0.454	0.008	0.642	0.040	0.234
Silt	0.202	**0.003**	<0.001	0.982	0.054	0.167
Clay	0.047	0.165	0.017	0.482	0.006	0.638
pH	0.096	**0.045**	0.008	0.639	0.005	0.674

Geographical Variables In all years, aspect and heat load did not have a statistically significant relationship with *Trifolium* spp. abundance (Table 1). However, slope and elevation did show relationships to *Trifolium* spp. abundance (Table 1). Slope had a positive relation, meaning *Trifolium* abundance increased with an increase in steepness (Figure 1). Furthermore, elevation also had a positive relationship, meaning *Trifolium* abundance increased with an increase in elevation (Figure 2).

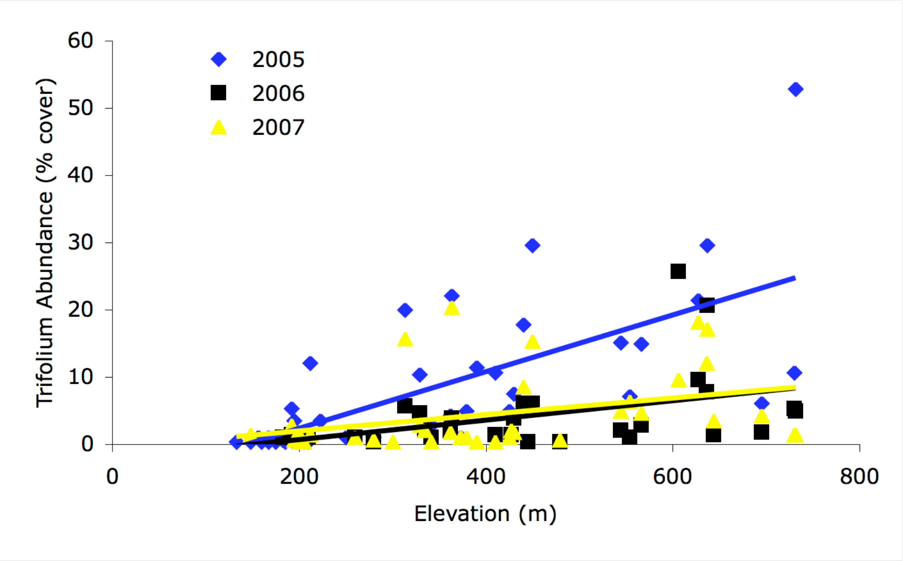

Figure 1: The relationship between *Trifolium* spp. abundance (% cover) and elevation (m) for 2005 (y=0.10x – 13, p=0.001), 2006 (y= 0.040x – 6.1, p=0.007), and 2007 (y=0.035 – 1.7, p=0.007), with sampling performed once between April and June of 2005, 2006, and 2007.

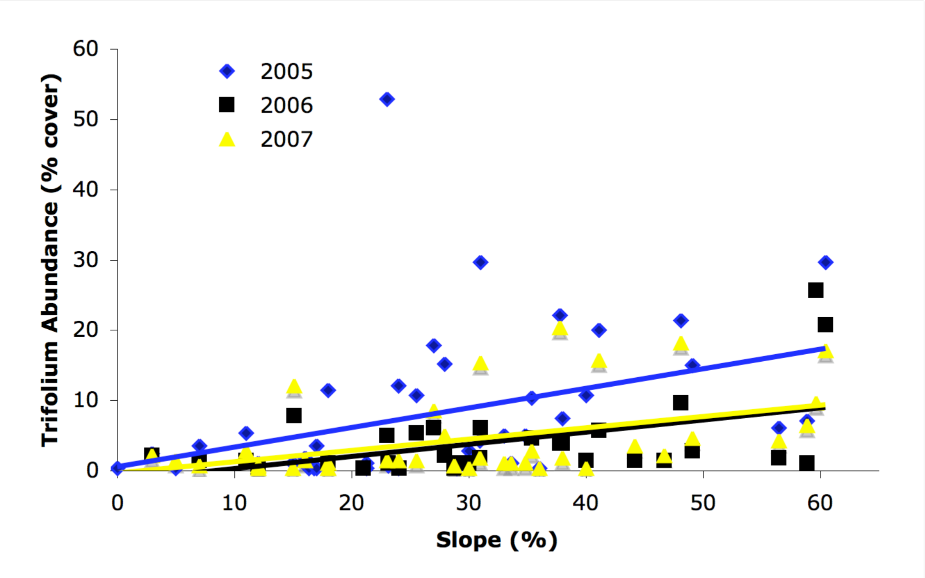

Figure 2: The relationship between slope (%) and *Trifolium* spp. abundance (% cover) for 2005 (y= 0.97x – 3.5, p=<0.001), 2006 (y= 0.49x – 4.1, p=0.014), and 2007 (y=0.46x – 1.4, p=0.032), with sampling performed once between April and June of 2005, 2006, and 2007.

Soil Composition In 2005, N total, C total, P, X-Ca, sand, and clay did not appear to have a relationship with *Trifolium* spp. abundance (Table 1). However, silt content had a positive relationship. *Trifolium* spp. abundance increased with an increased percentage of silt (Figure 3). Another variable, pH, had a negative relationship with *Trifolium* spp. abundance. As the pH level increased, the *Trifolium* spp. abundance decreased (Figure 4). In 2006 and 2007, P, X-Ca, sand, silt, clay, and pH were not determined to be statistically significant (Table 1). Significant results include N total and C total (Table 1). *Trifolium* spp. abundance decreased with an increase in the percentage of N total (Figure 5) and C total (Figure 6).

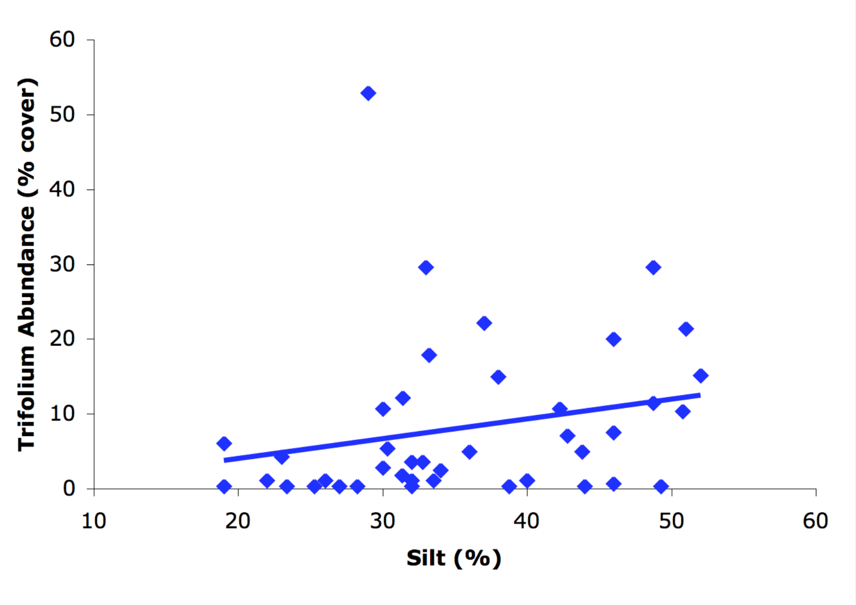

Figure 3: The relationship between silt (%) and *Trifolium* spp. abundance (% cover) for 2005 (y= 0.97x – 3.5, p=0.003), with sampling performed once between April and June.

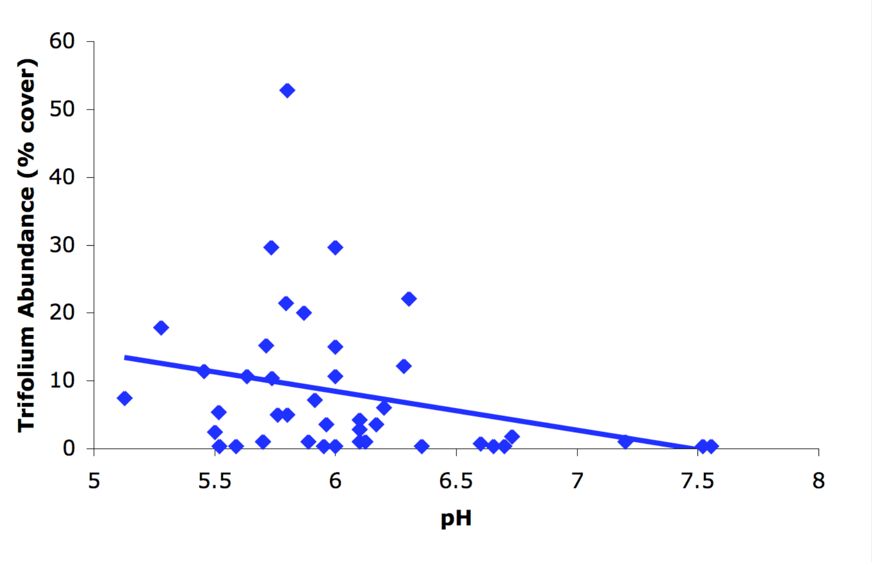

Figure 4: The relationship between pH and *Trifolium* spp. abundance (% cover) for 2005 (y= -16X + 120, p=0.045), with one sample taken between April and June.

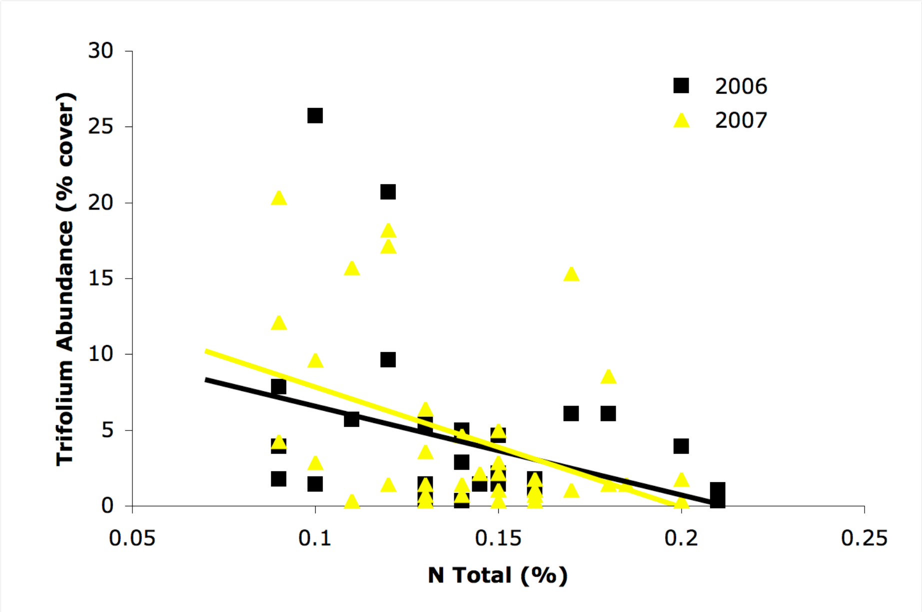

Figure 5: The relationship between N total (%) and *Trifolium* spp. abundance (% cover) for 2006 (y= -160x + 35, p=0.068) and 2007 (y= -230x + 46, p=0.008), with sampling performed once between April and June of 2006 and 2007.

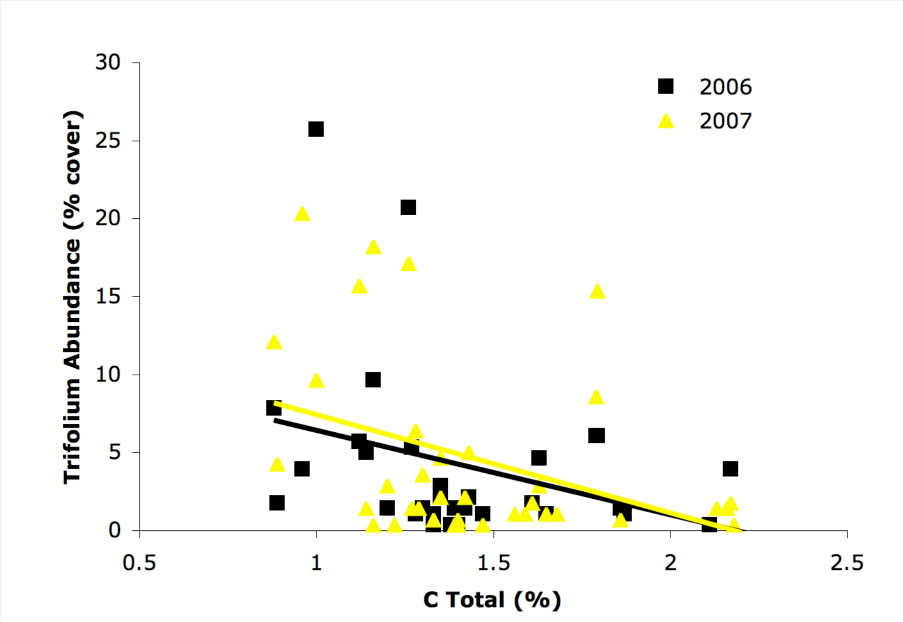

Figure 6: The relationship between C total (%) and *Trifolium* spp. abundance (% cover) for 2006 (y= -15x + 33, p=0.087) and 2007 (y= -18x + 39, p=0.016), with sampling performed once between April and June of 2006 and 2007.

Discussion

Of the *Trifolium* encountered, 72 percent of *Trifolium* hits were of a single nonnative species, *T. hirtum*. Therefore, the results are largely weighted by relationships between environmental variables and this species.

Geographical Variables This study found slope and elevation to relate to *Trifolium* spp. abundance (Table 1). *Trifolium* spp. abundance increased with elevation (Figure 1) in contrast with my hypothesis. However, the measurement of elevation may be slightly misrepresented. The greatest diversity was found at Sunol Regional Wilderness, averaging 200m higher than the other six parks sampled (533m vs. 332m respectively). Since this study did not have a stratified sample of elevations, Sunol may have weighted the results. This study found that *Trifolium* ssp. abundance increased with steepness (Figure 2). One possible explanation of this finding is that steeper slopes are the sites of more erosion, thus more disturbances. It is also possible that *Trifolium* are adept to disturbance and therefore more abundant at these locations. This study was unable to find past research dealing with either variable. However, based on observations, *Trifolium* were generally more abundant near areas of disturbance, such as along the edges of dirt roads. This may agree with one possible explanation discussed in this research and should be considered for future research.

Soil Composition In 2005, silt and pH showed relationships to *Trifolium* spp. abundance (Table 1). *Trifolium* abundance increased with an increase in the percentage of silt (Figure 3). This was unexpected and remains understudied in literature on *Trifolium* spp. This study found *Trifolium* abundance to decrease with an increase in pH level or basicity (Figure 4). This negative relationship coincides with the findings of George and Clawson (1987) who found that *Trifolium* spp. are more abundant in neutral to acidic soils. Through 2006 and 2007, N total and C total both maintained relationships to *Trifolium* spp. abundance (Table 1). *Trifolium* abundance decreased with an increase in the percentage of N (Figure 5), which agrees with the hypothesis of this study. Jones (1966) reaffirmed this by finding that N brings important changes to the botanical and chemical composition of grasslands. Furthermore, Jones and Winans (1967) found that the percentage of clover was reduced where N was applied. Another finding of this study was a relationship between *Trifolium* spp. and C total (Table 1). This study found that *Trifolium* abundance decreased with an increase in the percentage of C (Figure 6). This finding was an unforeseen association to *Trifolium* spp. and should be considered for future research.

Additional studies have dealt with phosphorus in relation to *Trifolium* spp. abundance. This study found no relationship with P and *Trifolium* spp. abundance (Table 1). However, past research has documented that the percentage of *Trifolium* spp. was increased by the addition of P (Jones and Evans 1960). Echoed by this finding, Conrad (1950) observed that P increased the total biomass of clovers and thus substantially increased the succeeding amounts of other vegetation.

This study found variations in the strength and significance of environmental relationships with *Trifolium* abundance. Within this study, analysis is limited because the soil and geographical variables were sampled only in 2006 and compared to *Trifolium* data from 2005, 2006, and 2007. Therefore, temporal changes, especially in soil variables, were not captured. Another factor that could contribute to the differences between years are the precipitation patterns. During the three-year period, precipitation ended at approximately the same time of the year, April or May. However, the first onset of ecologically meaningful rain varied in timing. In 2005, this occurred in October, while in 2006 and 2007 this occurred in December. This could have affected the timing of germination and growth rates for *Trifolium* seeds. George et. al. (1988) found that the timing and duration of rain can influence forage species, including *Trifolium*. This finding coincides with the findings of this study. *Trifolium* were more abundant in 2005, accounting for six percent of overall vegetation sampled compared to two or three percent in the other years of this study. It is possible that this early onset of precipitation results in more *Trifolium* and should be studied further.

While this study provides important background for the relationship between *Trifolium* spp. and environmental variables, more research can be performed. For example, an experimental component could determine the levels at which *Trifolium* is most abundant. Experimental studies testing the range of tolerance would greatly complement the observational study of this research. Furthermore, additional research could be conducted in other areas to determine if these variables are specific to the East Bay or general to all rangeland ecosystems. Additionally, one variable that has been shown to relate to *Trifolium* is sulfur, which was not tested for this study. Including this component would be another interesting variable to consider when looking for relationships between *Trifolium* spp. abundance and different environmental variables. Finally, in conducting future research, it should be cautioned that use of point intercepts is not as effective at detecting rare species as other methods, such as visual surveys. This limitation in design should be corrected in future studies to accommodate rare species of *Trifolium*. This study provides groundwork for future research, but it should be expanded and manipulated to further understand the clover community.

In conclusion, this study found *Trifolium* spp. to relate to steeper slope, higher elevations, and a decreased content of C and N. Understanding these relationships is important for the management of rangelands. *Trifolium* are legumes that fix atmospheric N and provide forage for grazing animals. Therefore, recognizing the variables that constitute a higher abundance of *Trifolium* spp. improves the overall quality of grasslands, including those in the East Bay. Theoretically, this study allows rangeland ecologists to improve grasslands through the addition of vegetation that restores depleted N on appropriate sites. More practically, this research provides background for the development of management practices that can aid in the spread of these ecologically important species.

Acknowledgements

I would like to thank James Bartolome for his constant guidance, literature, and providing me the opportunity to be a part of the Range Ecology lab. The lab, including Michele Hammond and Peter Hopkinson, has helped me in many ways from topic development and data collection to entertainment during the long field days. Finally, I would like to thank Pete Oboyski and Shelly Cole for their input and direction. This study could not have been conducted without the assistance of these people and I am forever grateful for their help.

References

Baker, H.G. 1989. Sources of the naturalized grasses and herbs in California grasslands. In Grassland structure and function: California annual grassland. L.F. Huenneke and H.A. Mooney, eds. Dordrecht, Kluwer Academic Publishers. 29-38.

Bentley, J.R. 1946. Range fertilization – one means of improving range forage. California Cattleman 9: 6-24.

Biswell, H.H. 1956. Ecology of California grasslands. Journal of Range Management 9: 19-24.

Conrad, J.P. 1950. Sulfur fertilization in California and some related factors. Soil Science 70: 43-54.

George, M.R. and W.J. Clawson. 1987. Improving feed production with annual legumes. Range Science Report 10: 1-5.

George, M.R., K. Olson and J.W. Menke. 1988. Range weather: a comparison at three California range research stations. California Agriculture 42: 30-32.

Graves, W.L., W.A. Williams, C.E. Vaughn, C.E. Thomsen and M.B. Jones. 2001. Australian varieties improve pasture in long-term annual legume trials. California Agriculture 55: 60-63.

Green, L.R. 1959. Some effects of sulfur fertilization on nodulation and growth of annual range legumes. Forest Service, U.S. Department of Agriculture 156: 1-7.

Holland, V.L. and David J. Keil. 1995. California vegetation. In California vegetation. M. Barbour and J. Major, eds. Iowa, Kendall/Hunt Publishing Company. 11-72.

Jones, M. B. 1966. Fertilizing annual grasslands in California. Fertilization Solutions 10: 10-12,14.

Jones, M.B. and R.A. Evans. 1960. Botanical composition changes in annual grassland as affected by fertilization and grazing. Agronomy Journal 52: 459-461.

Jones, M.B. and S.S. Winans. 1967. Subterranean clover verse nitrogen fertilized annual grasslands: biological composition and protein content. Journal of Range Management 20: 8-12.

Jones, M.B. and R.G. Woodmansee. 1979. Biogeochemical cycling in annual grasslands ecosystems. The Botanical Review 45: 11-144.

Kuchler, A.W. 1964. Potential natural vegetation of the conterminous United States. Special publication (No. 36). New York, American Geographical Society. 4-9.

Love, R.M. 1985. Rose clover. In Clover Science and Technology. N.L. Taylor, ed. Wisconsin, American Society of Agronomy. 535-546.

Lulow, M.E. 2007. Restoration of California native grasses and clovers: the role of clipping, broadleaf herbicide, and native grass density. Restoration Ecology 43: 1-10.

McCune, B. and J.B. Grace. 2002. Chapter 3. In Analysis of ecological communities. D.W. Goodall ed. Oregon, MjM Software Design. 22-24.

Murphy, A.H., M.B. Jones, J.W. Clawson and J.E. Street. 1973. Management of clovers on California grasslands. Division of Agriculture and Natural Resources. California, Publication 2661: 2-19.

Ulyatt, M.H. 1980. The feeding value of temperate pasture. In Grazing Animals. F.H.W. Morley, ed. Amsterdam, Elsevier Scientific Publishing Company. 125-141.

Weitkamp, B. and W. Graves. 1987. Annual Medics for range and grain lands. Davis, Department of Agronomy and Range Science 9: 1-6.