USING THE LICHEN XANTHOPARMEILA TO MONITOR CONCENTRATIONS OF NITROGEN IN URBAN AND WILDERNESS ENVIRONMENTS

Joseph Arehart
Email: jarehart13@gmail.com
Phone: 303-494-8116

Abstract:

In Colorado, there is a need to monitor and establish baselines to describe the normal concentrations of different pollutants in the atmosphere. By establishing baselines throughout different environments, we can monitor the impact of human activity on the atmosphere. While monitoring these changes can be done in many ways, the use of geographic information systems (GIS) makes monitoring levels of pollutants easier. Lichens have been used as bioindicators to monitor pollutants in the atmosphere as lichen absorb chemicals through wet (from precipitation) and dry (from the ambient air) deposition. Through a chemical analysis of nitrogen concentrations in the tissues of the lichen genus *Xanthoparmelia*, I compared the total percentage of nitrogen in lichen from both urban and wilderness environments to see if the geographic setting and distance from an urban center affects the levels of nitrogen found within lichen samples. Foliose lichen samples (n=86) were collected from rocks in both the Indian Peaks Wilderness Area (wilderness), as well from the Boulder County Open Space Areas (urban). After samples were collected and prepared, chemical analyses for nitrogen were conducted using a carbon-hydrogen-nitrogen (CHN) analyzer. The average total nitrogen levels contained in the lichen of the wilderness sites (1.116%) were significantly lower than those of the urban sites (1.422%, $p < 0.001$). However, the urban vs. wilderness differences were not readily explained by the factors of urban distance and elevation (a proxy for precipitation which accounts for the wet deposition in an area). These results established a preliminary baseline which can be used to monitor changes in Boulder County's "nitrogen footprint" over time.

Introduction:

In many ecosystems, baseline levels of pollutants need to be quantified. The monitoring of pollutants is a key component in identifying changes in the levels of pollutants which can be detrimental to ecosystems (Krebs, 2010). Lichens are symbiotic rootless organisms that obtain essential nutrients through wet and dry deposition. Through this same process, lichens absorb pollutants from the atmosphere which are stored in lichen tissues (McCune and Geiser, 2009; Garty, 2001). The lichen genus *Xanthoparmelia* is classified as a foliose lichen that is abundant throughout the Rocky Mountains and has been used in the study of Jackson et al. (2009), which focused on northwestern regions within Colorado (Corbridge and Weber, 1998). Lichens have been used throughout history to monitor the health of the atmosphere through both chemical analyses and community analyses (McCune and Geiser, 2009; Blett et al., 2003). The porous membrane of the lichen thalli makes lichen a viable organism for monitoring concentrations of pollutants (Garty, 2001). By monitoring the levels of particular pollutants, disruptions in nutrient cycles within ecosystems can be identified and monitored.

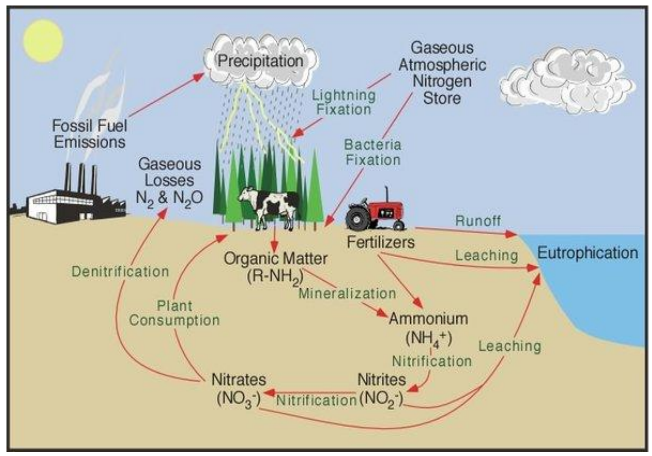

Figure 1. Nitrogen cycle showing the process by which nitrogen is cycled throughout an ecosystem with anthropogenic sources. Lichens absorb reactant forms of nitrogen from the air such as nitrites and nitrates, while not absorbing the inert gaseous atmospheric nitrogen (N_2). Figure from (http://www.eoearth.org/files/120901_121000/120944/600px-Nitrogencycle.jpg).

Nitrogen is an essential element needed by living organisms. The nitrogen cycle is a nutrient cycle that circulates nitrogen in different compounds through and across ecosystems. Living organisms need elemental nitrogen, yet most nitrogen is found in its gaseous state of N_2 which is inert and can only be used by nitrogen-fixing organisms. Because of this limitation, most organisms must obtain nitrogen from other more reactive forms such as ammonium or nitrate. Figure 1 describes the nitrogen cycle and the anthropogenic sources that have increased significantly during the past half century due to the use of fertilizers, livestock farming, the expansion of suburban areas, and car emissions (Galloway and Cowlin, 2002; Howarth et al., 2002). These sources of nitrogen are not inert and reside in reactive forms which can have environmental consequences (Fenn et al., 2003; Krebs, 2010). When reactive forms of nitrogen are released into the atmosphere, they are highly mobile and have the ability to travel great distances (Krebs, 2010). In addition, reactive forms of nitrogen can enter and disrupt the nitrogen cycle of an ecosystem. Seen across the western United States, this disruption of the nitrogen cycle can have adverse effects on an ecosystem's soil, water quality, plant growth and biodiversity (Fenn et al, 2003; Baron et al., 2000; Bowman et al., 2006). These changes in the nitrogen cycle begin to occur when levels of nitrogen in an ecosystem reach a certain point called the "critical load" (Bowman et al. 2006).

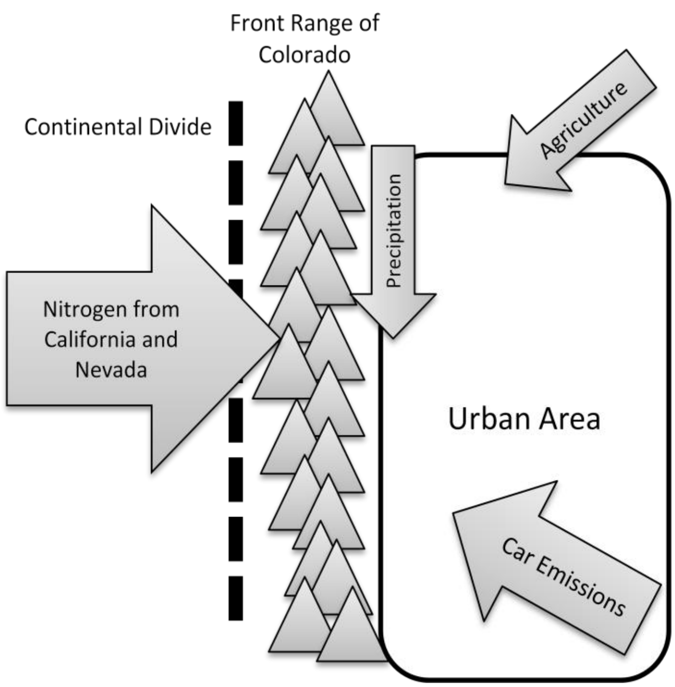

Figure 2. Anthropogenic sources of nitrogen that possibly affect the concentration levels found in lichen samples found along the Colorado Front Range.

Along the Colorado Front Range and in the Rocky Mountain region, there have been several studies conducted that have quantified the levels and critical loads of nitrogen in ecosystems. These studies have also sought to identify the sources of pollution that cause excessive levels of nitrogen within an ecosystem, thus affecting its biodiversity (Baron et al., 2000; Bowman et al., 2006).

Factors that may contribute to nitrogen deposition across the Front Range include agricultural production, car emissions from the Denver metro area, precipitation levels, and long range deposition of nitrogen (Fenn et al., 2003; Krebs, 2010). Figure 2 shows these anthropogenic sources which are possible factors that affect nitrogen levels. From the Denver-metro area, there are high concentrations of NO_x emissions from cars as well as deposition from the agricultural activity to the north (Beem et al., 2010). In the Rocky Mountains, sites on the eastern side of the Continental Divide have higher concentrations of nitrogen compared to the western side of the Divide, suggesting that proximity to anthropogenic sources in the urban and agricultural regions east of the Front Range contribute to the increased levels of nitrogen in aquatic as well as terrestrial ecosystems along the Front Range (Baron et al., 2000). An alternate hypothesis that explains the effects of nitrogen deposition in the Rocky Mountains is that nitrogen is being deposited through long-range deposition from California and Nevada (Krebs, 2010). In addition, levels of reactive nitrogen have been increasing significantly in Rocky Mountain National Park (RMNP) and were measured to be 20 times the normal background levels (Beem et al., 2010). These significant increases in nitrogen has prompted federal and state government agencies to develop the Rocky Mountain Nitrogen Deposition Reduction Plan to reduce Colorado's output of reactive nitrogen (Krebs, 2010).

In Boulder County, Colorado, there is a need to establish baselines levels of different elements and compounds such as nitrates, nitrites, sulfites, sulfates, mercury, as well as lead and other heavy metals. All of these elements and compounds are toxic to natural environments, and have the possibility to disrupt key cycles that keep the flow of nutrients in an ecosystem in balance.

Because of the increased levels of deposition in the Rocky Mountains including along the Colorado Front Range, monitoring of nitrogen and other pollutants needs to be carried out in order to quantify changes in pollution levels. The purpose of this study is to use a chemical analysis of nitrogen concentrations in the tissues of the lichen genus *Xanthoparmelia* to compare the total percentage of nitrogen in lichen from both urban and wilderness environments to see if the geographic setting and distance from an urban center affects the levels of nitrogen found within lichen samples.

Methods:

The lichen genus *Xanthopermelia* is abundant throughout the foothills of the Rocky Mountains as well as in Boulder County which is why it was used in this study (Jackson et al., 1996, Corbridge, 1998). From the urban environment (around the city of Boulder), 63 samples were collected from varied locations, as well as 23 samples from the wilderness environment (in the Indian Peaks Wilderness).

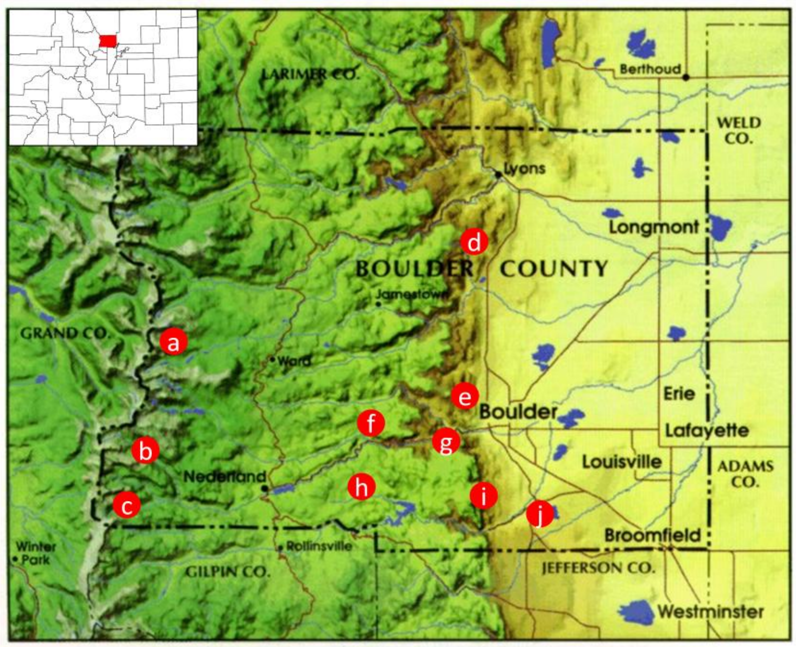

Figure 3. Map of Boulder County with collection sites marked: (a) Long/Mitchell Lake, (b) 4th of July, (c) Betty Lake, (d) Heil Valley Ranch, (e) Mt. Sanitas, (f) Betasso Preserve,(g) Gregory Canyon, (h) Walker Ranch, (i) Shanahan Ridge, and (j) Marshall Mesa. Sites a through c are wilderness sites while d-j are classified as urban sites. Map taken from http://www.boulder.doc.gov/vis-info/visiting.html

Figure 4. *Xanthoparmelia* lichen on a rock. Samples like this were collected at every site.

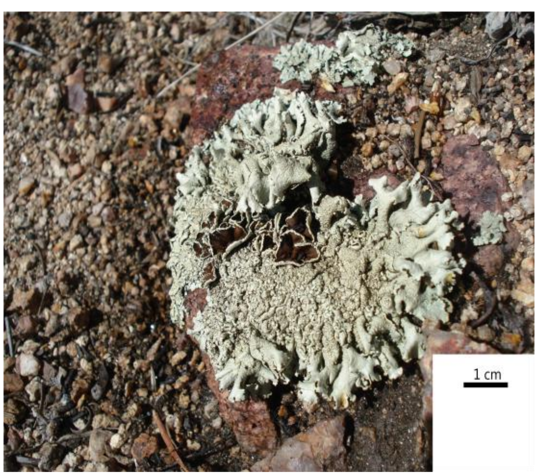

Figure 3 shows the locations of each collection site in Boulder County. Sites (a), (b), and (c) are wilderness sites while sites (d), (e), (f), (g), (h), (i), and (j) are urban sites. Collection locations were varied throughout the area of each site, and approximately 8 samples were taken at each site (both wilderness and urban). The exceptions were as follows: seven samples were collected from the Betty Lake and 4th of July sites in the wilderness environment and 16 samples were collected from Betasso Preserve. Because of the Fourmile Canyon Fire which occurred on September 7th of

2010, 16 samples were collected at this urban site which is in close proximity to the location of the wildfire. More samples were collected at this site to determine if any immediate effects of the Fourmile Canyon Fire could be measured by analyzing the lichen tissues. The samples were collected between July and October of 2010. To denote the location of each sample collected, a Garmin brand eTrex Vista HCx Global Positioning System (GPS) was used to capture the coordinates of each sample. The environment of the wilderness and urban areas can be seen in Figure 5, which shows photographs of examples of the habitats from which lichen samples were collected.

Figure 5. Wilderness habitat on the left and urban habitat on the right. These habitats were the average across all sites.

The locations of the urban and wilderness sites stretch across Boulder County (refer to Figure 1). The wilderness sites are close to the edge of the county line as well as the Continental Divide, while the urban sites surround the city of Boulder as well as agricultural areas. A GPS was used to determine the mean distance from each site to the urban center (Valmont Dike). These mean distances are listed in Table 1.

Table 1. Mean Urban Distance in kilometers from each site to Valmont Dike the urban center.

Site	*Mean Urban Distance (km)*
Betty Lake	39.68
Long/Mitchell Lake	33.39
4th of July	36.62
Betasso Preserve	11.21
Walker Ranch	13.71
Marshall Mesa	9.25
Heil Valley	15.31
Mt. Sanitas	7.44
Gregory Canyon	8.14
Shanahan Ridge	8.47

At approximately four locations within a site area (except for the exceptions listed above), two foliose lichen individuals such as the one seen in Figure 4 were identified to the genus *Xanthoparmelia* and scraped off of a rock using a plastic card. After collection, the samples were stored in paper bags and taken to the Bowman Laboratory at the University of Colorado at Boulder to be processed for analysis of nitrogen. In the lab, the lichen samples were dried in a convection oven at 60° C and then were ground in inert liquid nitrogen with a mortar and pestle. Rubber gloves were worn at all stages of the process to ensure that no foreign particulates contaminated the lichen sample. The ground-up lichen samples were then weighed into aluminum tins that were then analyzed by a carbon-hydrogen-nitrogen (CHN) analyzer. The CHN analyzer combusts the tin with the ground up lichen samples and measures the total percentage of nitrogen within the sample.

The total nitrogen levels were then compared across sites and a t-test was used to test for variance between both urban and wilderness sites. Factors that might have contributed to the variances between urban and wilderness sites were then looked at using linear regression models.

Geographic Information System (GIS) analysis was carried out to visualize how concentrations of nitrogen in lichen samples varied across the geography of the Front Range. By using a GPS system, the data was mapped onto an aerial photograph taken by Google. Since I did not have access to commercial GIS software during this study, I used the free program GPS Visualizer which I found at www.gpsvisualizer.com. This program was used to plot geographic information taken from a GPS system as well the concentrations of nitrogen at each of the sites. By creating circles superimposed onto a satellite image, conclusions could be drawn because the different sized circles represented different concentrations on nitrogen at each site. For definitions of terms used in this study refer to Appendix A.

Results:

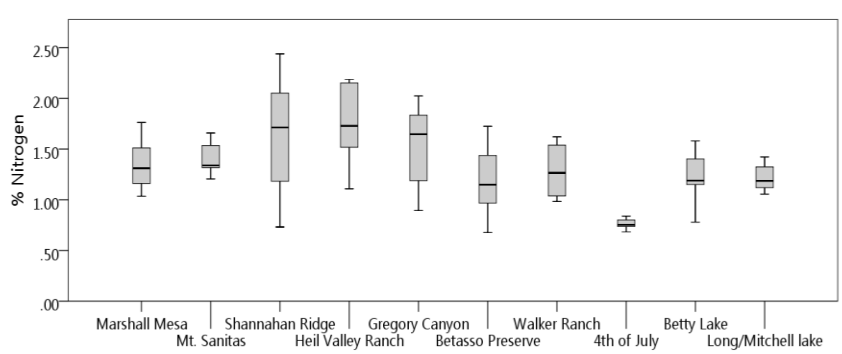

Figure 6. Box Plot of each individual site (7 urban and 3 wilderness) demonstrating the range of nitrogen levels within a site and variability across sites. In each box, the horizontal line represents the median; the error bars represent the minimum and maximum levels, while the top and bottom of the boxes represents the upper and lower quartiles.

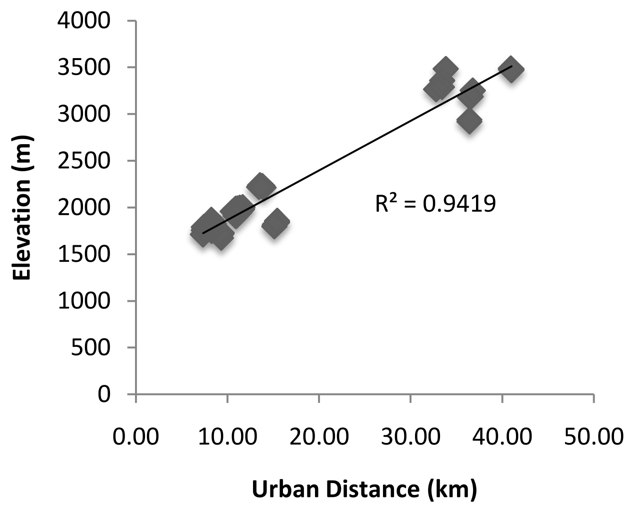

Figure 7. One objective of the analysis was to explore the possible association between nitrogen levels and a) the distance between each sample and the urban center (Valmont Dike) and b) the precipitation levels which account for the wet deposition in a site.

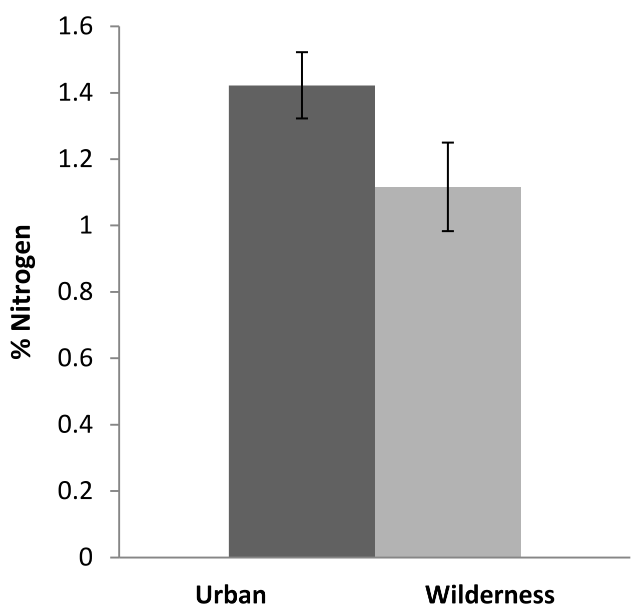

Figure 8. Average values of percent nitrogen from Urban and Wilderness sites (1.422% and 1.116% respectively). Error bars represent 95% Confidence Intervals (0.0998 and 0.1333 respectively). There is a significant difference between the urban and wilderness sites as the error bars do not intersect with one another.

The concentrations of nitrogen among lichen samples varied greatly from site to site as shown with the box plot in figure 6. The site with the most variability was Shanahan Ridge, while the site with the least amount of variance was 4th of July. To answer the question of whether the wilderness environments had different concentrations of nitrogen than urban environments, a t-test of the data was preformed. The t-test showed that the differences between urban and wilderness sites was significant (p <.0.01, n=86). Factors that might have contributed to the differences of nitrogen levels between urban and wilderness sites were urban distance as well as elevation. However, as shown in Figure 7, urban distance and elevation are highly correlated (R^2=0.9419). Therefore it is difficult to distinguish between each of these factors as the primary cause of the increase in nitrogen levels. Elevation can be used as a proxy for precipitation, which accounts for the wet deposition that nitrogen receives, as higher precipitation levels occur at higher elevations.

Figure 9 shows the correlation between total percent nitrogen and elevation. No correlations between elevation and total nitrogen can be made as there are relatively small Pearson correlation coefficients for both the urban as well as the wilderness settings (0.2187 and 0.0455 respectively).

GPS visualizer, a GPS visualization software available on the internet (found at http://www.gpsvisualizer.com/), gives geographic representations of data by plotting different sized circles correlated with GPS coordinates onto a map using Google Earth. These data plots are shown in Figures 10a and 11. Figure 11 shows the concentrations of nitrogen from each site. The relative size of the circle represents the relative concentrations of nitrogen within a site, so larger circles represent higher nitrogen levels. As the distance between the site and the city of Boulder increases, the size of the circle goes down which is represented geospatially in Figure 11. Figure 11 shows the geographic representation of the concentrations of nitrogen in the Betasso Preserve, an urban site. Because of the Fourmile Fire, which occurred in the Fall of 2010, this site was looked at in more depth in order to see if there were any immediate differences in concentrations of nitrogen close to the burn area compared to concentrations at locations farther away. Visual inspection of the map showed no immediate differences between the sites close and far away. However further study of this region needs to be done in order to monitor levels of nitrogen.

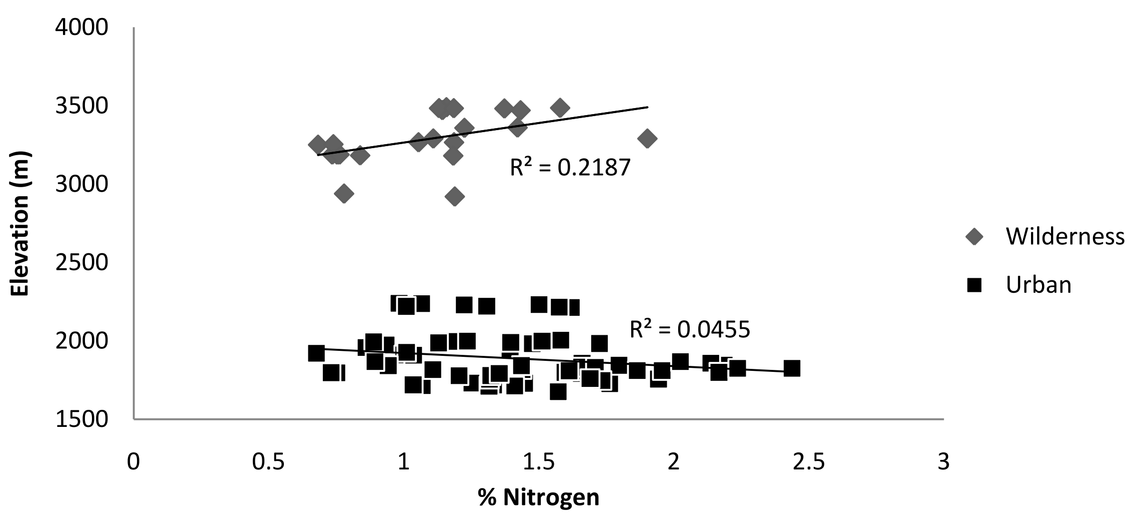

Figure 9. % Nitrogen vs. elevation for urban and wilderness sites with linear lines of best fit showing possible trends that as elevation decreases for urban sites, the levels of % nitrogen decreases (R^2 = 0.0455), while at high elevations (above 2500m) as the elevation increases, so do the concentrations of nitrogen (R^2 = 0.2187).

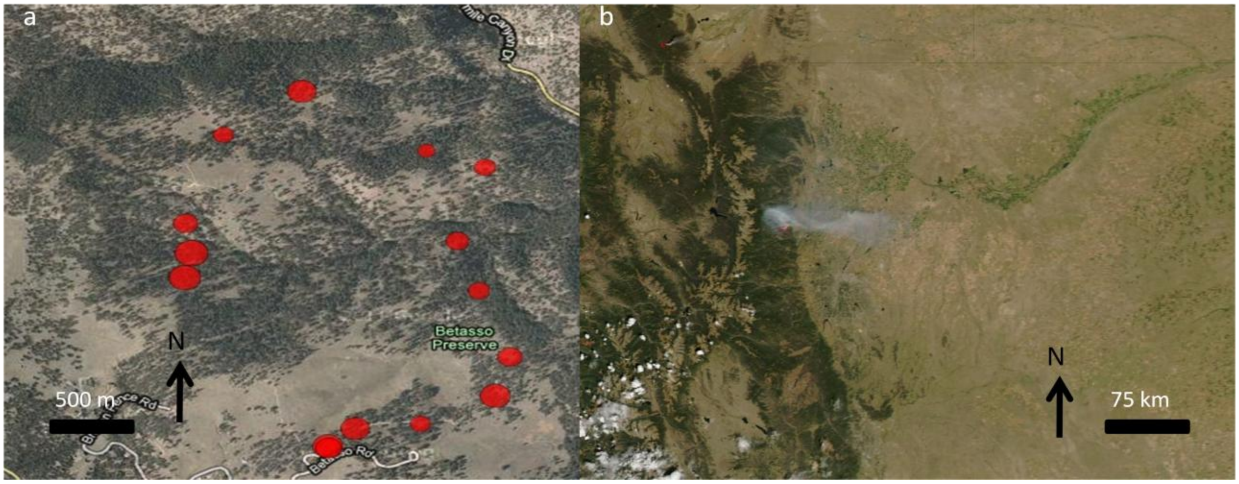

Figure 10. a) Nitrogen plotted over a satellite image for the Betasso Preserve. The Fourmile Fire was to the northwest of this collection site, and no conclusions about the fire's nitrogen impact can be made as the sizes of the circles (concentrations of nitrogen) do not show systematic trends b) is an aerial photograph taken NASA's Aqua Satellite which was able to see visible smoke across Boulder County as well as the northeastern region of Colorado.

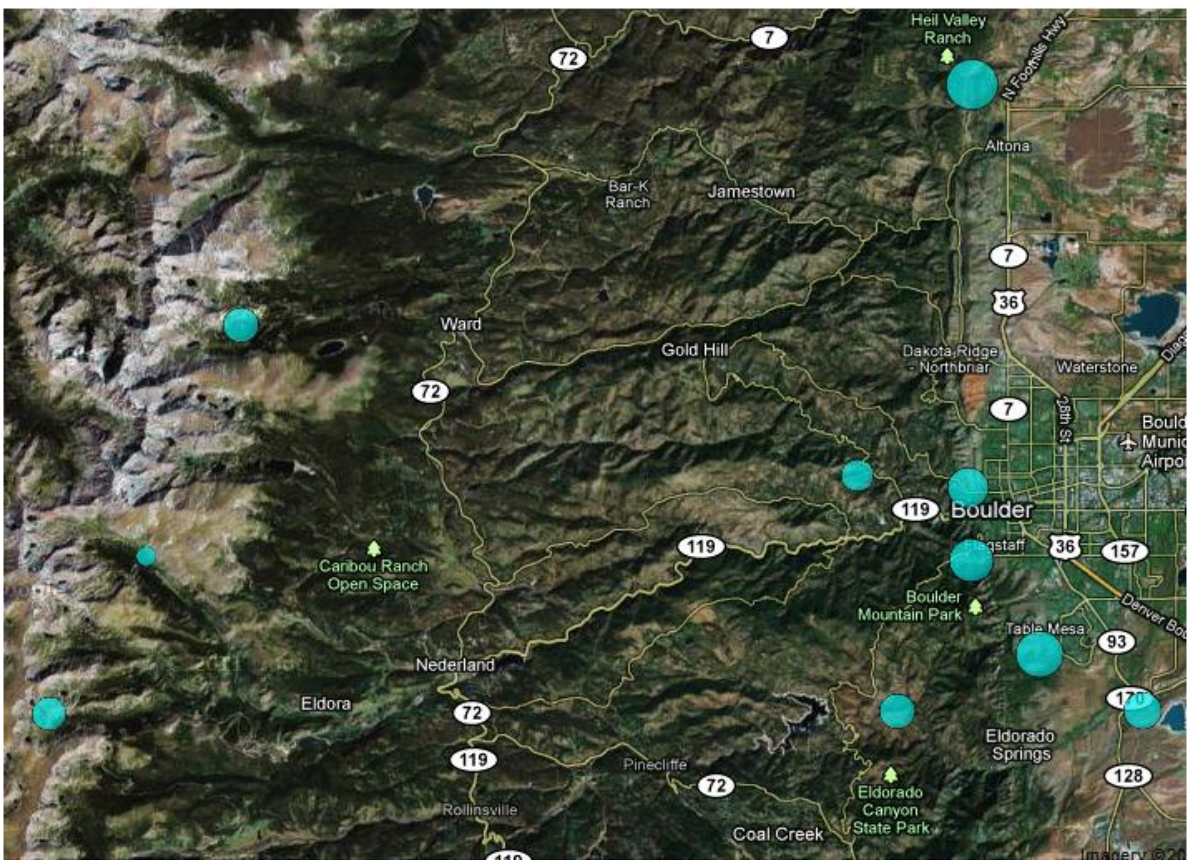

Figure 11. This map shows the concentrations of nitrogen (indicated by the relative size of the circle) at each of the 10 collection sites using GPS visualization software (http://www.gpsvisualizer.com/).

Discussion:

The present results are mixed in terms of answering the primary research question that asked how distance from urban areas affects concentrations of nitrogen in the tissues of the lichen *Xanthoparmelia*. The initial *t*-test supports the prediction that the concentrations of nitrogen are higher in urban environments. However, the urban vs. wilderness differences are not readily explained by the factors of urban distance and elevation (as a proxy for precipitation which accounts for the wet deposition that an area receives). Additional monitoring with data visualization will help to find changes in particular areas of Colorado.

The design of this study has several limitations including the relatively small sampling size, the differences in habitat across the sites, and the focus on a single species of lichen. Further and more extensive sampling is needed throughout Boulder County, as well as along the Front Range to monitor the “nitrogen footprint” that Boulder County is making. Isolating factors such as elevation within a sampling site as well as urban distance would possibly provide more insight into the specific sources of anthropogenic nitrogen.

Recent studies have suggested that reactive nitrogen found in Rocky Mountain National Park and along the Front Range of Colorado can be attributed to multiple sources including nitrogen pollution from California and Nevada and from agricultural and urban areas on the plains of Colorado (e.g. Krebs, 2010). An extension of this study in the local area surrounding Boulder County would be to look at the differences in nitrogen levels from the East and West sides of the Continental Divide and see if there is a gradient of increasing nitrogen levels across the Rocky Mountains.

To monitor the “nitrogen footprint” of Boulder County, consistent monitoring of nitrogen levels across varied sites in the county needs to be carried out. In addition, more research is needed to pinpoint the locations from which anthropogenic reactive nitrogen sources are being emitted.

Identifying these locations will provide evidence to guide government regulations which are aimed at minimizing human-caused imbalances in ecosystems.

Conclusion:

This study quantified the levels of nitrogen in urban and wilderness areas within Boulder County. The results showed significantly higher levels of nitrogen in the seven urban sites sampled versus the three wilderness sites samples. The results establish a preliminary baseline that can be used to monitor changes in Boulder County's "nitrogen footprint" over time. Such monitoring is important to determine if anthropogenic sources of nitrogen continue to adversely affect Rocky Mountain ecosystems or if the effects are being mitigated through efforts such as the Rocky Mountain Nitrogen Deposition Reduction Plan.

In addition, other monitoring needs to be done in other places in Colorado and the western United States. Since reactive forms of nitrogen are highly mobile and can travel great distances, deposition along the Front Range might not only be caused by local anthropogenic sources but also sources from California and Nevada. Limited conclusions from this study can be made at this time, so more extensive monitoring needs to be done to test for changes in nitrogen and other elements in Boulder County and across the Western United States.

References:

Baron, J. S., Rueth, H. M., Wolfe, A. M., Nydick, K. R., Allstott, E. J., Minear, J. T., et al. (2000). Ecosystem responses to nitrogen deposition in the Colorado Front Range. *Ecosystems, 3*(4), 352-368.

Beem K., Raja S., Schwandner F., Taylor C., Lee T., Sullivan A., Carrico C., McMeeking G., Day D., Levin E., Hand J., Kreidenweis S., Schichtel B., Malm W., Collett J., (2010) Deposition of reactive nitrogen during the Rocky Mountain Airborne Nitrogen and Sulfur (RoMANS) study. *Environmental Pollution*, 158 (2010) 862-872.

Benedict, J. B. (2008). Experiments on lichen growth, III. The shape of the age-size curve. *Arctic Antarctic and Alpine Research, 40*(1), 15-26.

Blett, T.L. Geiser, and E. Porter. (2003). Air pollution-related lichen monitoring in national parks, forests, and refuges: Guidelines for studies intended for regulatory and management purposes. National Park Service Air Resources Division, Denver, CO. NPS D2292. http://www.nature.nps.gov/air/Pubs/pdf/Lichen_Studies.pdf

Brodo, I.M., Sharnoff, S.D., and Sharnoff, S. (2001) *Lichens of North America*. Yale University Press, Connecticut.

Bowman W., Gartner J., Holland K., Wiedermann M. (2006). Nitrogen Critical Loads for Alpine Vegetation and Terrestrial Ecosystem Response: Are we there Yet?., *Ecological Application* 16(3), 1183-1193.

Corbridge J., Weber W., (1998) *A Rocky Mountain Lichen Primer*. University Press of Colorado, Colorado.

Fenn M., Baron J., Allen E., Rueth H., Nydick K., Geiser L., Bowman W., Sickman J., Meixner T., Johnson D., Neitlich P., (2003). Ecological Effects of Nitrogen Deposition in the Western United States. *Bioscience*, *53* (4), 404-420.

Flock, J. W. (1978). Lichen-Bryophyte distribution along a snow-cover-soil-moisture gradient, Niwot-ridge, Colorado. *Arctic and Alpine Research, 10*(1), 31-47.

Glavich, D. A., & Geiser, L. H. (2008). Potential approaches to developing lichen-based critical loads and levels for nitrogen, sulfur and metal-containing atmospheric pollutants in North America. *Bryologist, 111*(4), 638-649.

Gadsdon S., Dagley J., Wolseley P., Power S., (2010). Relationship between lichen community composition and concentrations of NO_2 and NH_3. *Environmental Pollution*, *158* (2010) 2553-2560.

Galloway J., Cowling E., (2002). Reactive Nitrogen and the world: 200 years of change. *Ambio*, 31, 64-71.

Garty J. (2001). Biomonitoring Atmospheric Heavy Metals with Lichens: Theory and Application. *Critical Reviews in Plant Science*, *204* (4), 309-371.

Howarth, R., Boyer E., Pabich W., and Galloway J., (2002). Nitrogen use in the United States from 1961-2000 and Potential Future Trends. *Ambio* 31, 88-96.

Jackson, L., L. Geiser, T. Blett, T. Gries, and D. Haddow. 1996. Biogeochemistry of Lichens and Mosses in and Near Mt. Zirkel Wilderness, Routt National Forest, Colorado: Influences of Coal Fired Power Plant Emissions. *USDOI U.S. Geological Survey*.

Jovan S., McCune B., (2005). Air-Quality bioindication in the Greater Central Valley of California, with Epiphytic Macrolichen Communities. *Ecological Society of America*, *15* (5), 1712-1726.

Krebs Candace (2010). Unusually high nitrogen levels found in Colorado's Front Range. *Ag Journal*. 31 December 2010

Nimis, P., Scheidegger D., & Wolseley P. (Eds.). (2002). *Monitoring with Lichens- Monitoring Lichens* (Vols. 7) Dordrecht, The Netherlands: Kluwer Academic Publishers.

McCune, B., & Geiser, L., (2009). *Macrolichens of the Pacific Northwest*. Oregon State University Press, Corvallis.

Onipchenko, V. G., Blinnikov, M. S., Gerasimova, M. A., Volkova, E. V., & Cornelissen, J. H. C. (2009). Experimental comparison of competition and facilitation in alpine communities varying in productivity. *Journal of Vegetation Science, 20*(4), 718-727.

Pidwirny, M. (2006). "The Nitrogen Cycle". *Fundamentals of Physical Geography, 2nd Edition*. http://www.physicalgeography.net/fundamentals/9s.html

Raymond B., Bassingthwaighte T., Shaw D., (2010). Measuring nitrogen and sulphur deposition in the Georgia Basin, British Columbia, using lichens and moss. *Environment Canada*, *69* (1), 22-32.

Rogers, P. C., & Ryel, R. J. (2008). Lichen community change in response to succession in aspen forests of the southern Rocky Mountains. *Forest Ecology and Management, 256*(10), 1760-1770.

Smith Yvette (Sept. 7, 2010) "Fourmile Canyon Fire Aerial Photograph". Taken from NASA/MODIS's Aqua satellite. Viewed at http://www.nasa.gov/multimedia/imagegallery/image_feature_1754.html

Appendix A:

Definitions of terms used in this study are as follows. The term “Front Range” refers to the edge of the Rocky Mountains to the east of the Continental Divide in the state of Colorado. The term “environment” refers to either the wilderness or urban environment where samples were collected, while the term “site” refers to the 10 sites from which lichen were collected. See Figure 3.