THESIS

AN INVESTIGATION OF NITROGEN FIXATION BY RUSSET BUFFALOBERRY IN COLORADO CONIFER FORESTS

Submitted by

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ABSTRACT

AN INVESTIGATION OF NITROGEN FIXATION BY RUSSET BUFFALOBERRY IN COLORADO CONIFER FORESTS

Russet buffaloberry (*Shepherdia canadensis* (L.) Nutt.) is an actinorhizal shrub capable of forming a symbiotic relationship with the N_2-fixing soil actinomycetes *Frankia*. Actinorhizal shrubs are important species as they are able to fix an ecologically significant amount of N and can inhabit disturbed sites with infertile soils. Buffaloberry is commonly found as a dominant understory species in lodgepole pine (*Pinus contorta* Douglas ex Louden) communities and is a common post-fire disturbance species. There is a lack of information regarding buffaloberry's ability to fix atmospheric N_2 in Colorado forests. This study used the ^{15}N natural abundance method in a survey of buffaloberry in north central Colorado to determine the percent of foliar N that buffaloberry derives from fixation ($\%N_{dfa}$) and how fixation may be affected by local environmental factors. The mountain pine beetle (*Dendroctonus ponderosae*) epidemic is currently responsible for large losses in lodgepole pine forests. As the overstory canopies of lodge pole pine communities die off, there is an increase in available light in the understory. I investigated buffaloberry's response to light availability because with more photosynthetic activity, buffaloberry could potentially have more energy to expend in the energy intensive N_2-fixation process. 59 plots (0.1-ha) were sampled in July 2009 and were distributed among Larimer, Jackson, and Grand counties in Colorado. Buffaloberry (^{15}N: −0.63‰, N: 3.48%) had a ^{15}N abundance closer to the atmospheric standard with high foliar %N content as compared to non-N_2-fixing reference species (^{15}N: −1.29- −4.81‰, N: 1.11-3.20%), indicating biological N_2-

fixation. I estimate a probable range of foliar %N derived from biological fixation as 60-100%. Buffaloberry (2.65%) also had higher % foliar N as compared to the reference species (1.50%) in the autumn, just before leaf abscission. There were no significant correlations between light availability and N_2-fixation by buffaloberry suggesting that N_2-fixation in buffaloberry may not benefit from an increase in light availability.

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I would like to dedicate this degree, and this thesis, to the little light of my life, my daughter, Vitanie. I returned to complete my graduate degree for not only to provide a stable future for her, but more importantly to model for her how to follow your passion. She has such a beautiful, strong spirit that helped her, and me, get through the difficult weeks apart from her mama who was working in the field or trying to survive statistics class. Vitanie, thank you.

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INTRODUCTION

Actinorhizal plants can provide an ecologically significant amount of biologically fixed nitrogen (N) through a symbiotic relationship with N_2-fixing soil actinomycetes *Frankia* (Klemmedson 1979, Vitousek et al. 1987, Hibbs and Cromack Jr 1990). With their ability to inhabit soils with low N availability, actinorhizal plants are often early colonizers after disturbance and can be used in revegetation to aid in the development of nutrient poor sites (Klemmedson 1979, Huss-Danell 1997, Paschke 1997). The majority of studies conducted on actinorhizals in the western United States have shown significant levels of N derived from fixation (N_{dfa}) and inputs of ecosystem N, indicating the importance of actinorhizal shrubs for development in N-limited systems (Table 1).

Russet buffaloberry (*Shepherdia canadensis* (L.) Nutt.) is an actinorhizal shrub (Torrey 1978, Klemmedson 1979) distributed from northern Canada and Alaska south to New Mexico and Arizona and east from California to Maine. Aided by actinorhizal associations, buffaloberry has the ability to colonize disturbed areas and post-fire sites with low N availability (Klemmedson 1979). In addition, buffaloberry can maintain its prevalence in later-seral habitats (Walls et al. 2000), an unusual attribute for an N_2-fixing shrub, since most occupy early-seral, open sites such as newly formed dunes and floodplains. Buffaloberry is somewhat of a shade tolerant species and can be found under comparatively dense overstory stands (Uresk and Severson 1998, Walls et al. 2000). Since buffaloberry is found in many stages of succession, the berries are an important source of food for many forms of wildlife and it is occasionally a source of browse for wild ungulates (Walkup 1991).

Table 1. Some estimated values of N derived from biological fixation (N_{dfa}) and nitrogen accretion by a variety of actinorhizal shrubs in the western United States. Table modified from Paschke (1997).

Species	N_{dfa} (%)	Location	Reference
Buckbrush (*Ceanothus cuneatus* (Hook.) Nutt.)	36-69	Sequoia National Park, CA	(Shearer and Kohl 1986)
Chaparral whitethorn (*Ceanothus leucodermis* Greene)	45-95	Sequoia National Park, CA	(Shearer and Kohl 1986)
Prostrate ceanothus (*Ceanothus prostratus* Benth.)	20-44	California	(Busse et al. 2007)
Bitterbrush (*Purshia tridentata* (Pursh) DC.)	37-55	California	(Busse et al. 2007)
Snowbrush (*Ceanothus velutinus* Douglas ex Hook) and bitterbrush	>80	Oregon	(Busse 2000)
Species	**Total N fixed (kg ha^{-1} yr^{-1})**	**Location**	**Reference**
Snowbrush	95-100	Oregon	(Binkley et al. 1982)
Snowbrush	4-15	Central Oregon	(Busse 2000)
Snowbrush	70-108	Oregon	(Youngberg and Wollum 1976)
Snowbrush	101	Oregon	(McNabb and Cromack 1983)
Snowbrush	0-20	Oregon	(Zavitkovski and Newton 1968)
Curl-leaf mountain mahogany (*Cercocarpus ledifolius* Nutt.)	7	California	(Lepper and Fleschner 1977)
Bitterbrush	1	Central Oregon	(Dalton and Zobel 1977, Busse 2000)

Across North America, there have been a few studies investigating buffaloberry and its role in successional development (Kohls et al. 2003, Rhoades et al. 2008). Approximately 25 kg total N ha^{-1} yr^{-1} accumulated in soil on average when buffaloberry dominated the shrub community during the first 120 years of succession on a floodplain terrace in northwest Alaska (Rhoades et al. 2008). In southeast Alaska, there is evidence that three actinorhizal shrubs, including buffaloberry, provided the dominant source of N during the early stages of succession (Kohls et al. 2003). In British Columbia buffaloberry can fix an average of 9.36 kg N ha^{-1} yr^{-1} (Hendrickson and Burgess 1989). As demonstrated in these regions, buffaloberry provides a

source of biologically fixed N (Hendrickson and Burgess 1989, Kohls et al. 2003, Rhoades et al. 2008) similar to other actinorhizal shrubs (Table 1).

Buffaloberry is commonly found as a dominant understory species in lodgepole pine (*Pinus contorta* Douglas ex Louden) communities in Colorado. However, Colorado forests are rapidly changing due to die-off of lodgepole pine associated with the mountain pine beetle (*Dendroctonus ponderosae*). The Colorado State Forest Service estimates that two-thirds of all lodgepole pine forests in Colorado (404,767 ha) have been disturbed by the mountain pine beetle since 1996 (Ciesla 2009). As lodgepole pine communities are affected by the mountain pine beetle, changes in overstory canopy may affect buffaloberry as a dominant understory species and alter its N_2-fixing capacity.

There are several naturally occurring actinorhizal species in Colorado including buffaloberry, yet there have been few studies examining their N-fixing potential in this region. In Colorado it is unconfirmed if buffaloberry derives N from biological fixation and there has been no research on its contribution of N to surrounding soils by accretion. Because different environmental conditions such as phosphorus (P) availability, temperature (Houlton et al. 2008), and soil moisture (Sprent 1972) affect N_2-fixation rates, buffaloberry may have different N_2-fixation rates in Colorado as compared to other regions it inhabits.

N_2-fixation is an energy intensive process that can be negatively affected by a decrease in light availability (Bormann and Gordon 1984, Pastor and Binkley 1998, Rastetter et al. 2001, Vitousek et al. 2002, Houlton et al. 2008, Finzi and Rodgers 2009). There was a significantly positive correlation between photosynthetic rates and biological N_2-fixation as measured by acetylene reduction rates in European alder (*Alnus glutinosa* (L.) Gaertn) (Dawson and Gordon 1979), and red alder (*Alnus rubra* Bong.) demonstrated lower N_2-fixation rates in dense stands that limited photosynthate production (Heilman and Stettler 1983, Bormann and Gordon 1984). The negative relationship between N_2-fixation and light availability has also been demonstrated in studies with legumes (Sprent 1973, Khadka and Tatsumi 2006), which form a similar N_2-fixing

association with *Rhizobium* spp. As the overstory of lodgepole pine opens from die-off from the mountain pine beetle, the potential increase in photosynthate production may influence the amount of N_2-fixation by buffaloberry and may change the N economy of these systems.

N_2-fixation can be determined using the ^{15}N natural abundance method (Delwiche et al. 1979, Virginia and Delwiche 1982, Shearer and Kohl 1986, Högberg 1997). This method uses the assumption that if N_2-fixing plants derive and incorporate atmospheric N_2, the plant ^{15}N abundance will be closer to the atmospheric standard as compared to non-N_2-fixing species using other sources of plant available N. Using this method, N_2-fixation can be detected by measuring the isotopic $^{14}N/^{15}N$ ratio and document foliar ^{15}N levels in buffaloberry.

Using the ^{15}N natural abundance method and %N concentration analysis, I also was able to take a closer look at how the foliar ^{15}N and %N may change throughout a growing season in buffaloberry. N_2-fixation has been shown to be highest in the early months of the growing season in *A. rubra* (Bowman et al. 1996). Temperate trees and shrubs generally conserve foliar N by resorption before leaf senescence (Kolb and Evans 2002, Stewart et al. 2008), however N_2-fixing plants generally resorb proportionately less than non- N_2-fixing plants (Stewart et al. 2008). Because of the lack of research on buffaloberry, it is unknown if the foliar levels of ^{15}N and %N content are maintained at a constant level throughout the growing season.

With their high N content, the berries of buffaloberry are an important source of food for bears (*Ursus* spp.), snowshoe hares (*Lepus americanus*), and terrestrial birds across North America (Hamer 1996, Seccombe-Hett and Turkington 2008). In Canada, berry production decreased as forest canopy cover increased in fire-successional sites (Hamer 1996). In Colorado, the disturbance of the mountain pine beetle will initially open the canopy creating a higher light environment. With both potentially higher photosynthetic and N_2-fixing rates from more light availability, buffaloberry may be able to expend more energy on fruit production.

The first objective of this study was to determine if buffaloberry actively fixes N_2 through a symbiotic association with *Frankia* in Colorado forests using the ^{15}N natural abundance

method. Assuming that there would be active N_2-fixation by buffaloberry, the second objective was to identify if light availability affects N_2-fixation by buffaloberry and how it maintains the foliar ^{15}N and %N throughout the growing season. I hypothesized buffaloberry would respond similarly to other N_2-fixing species and that N_2-fixation would increase as light availability increases. The final objective of this study was to investigate if light availability also affects berry production by buffaloberry. I hypothesized that berry production would increase as light availability increases.

METHODS

Study Area Description

Plots were established in lodgepole pine forests of north central Colorado in July 2009. There were 59 plots, with one plot sampled repeatedly during the growing season from June to September. Plots were located in Jackson, Larimer, and Grand counties in the Arapaho, Roosevelt and Routt National Forests and the Colorado State Forest (Figure 1), roughly extending from 40°49' N, 105°51' W to 39°53' N,105°52' W. Elevation ranged from 2463 m to 3121 m. Ten year (200x – 200x) mean annual air temperature and precipitation across the study area ranged from 0.98 °C and 561.85 mm at the northern plots near Gould, CO, to 1.88 °C and 507.24 mm at the southern plots near Fraser, CO (HPRCC 2011). In 2009 mean annual air temperature and precipitation ranged from 0.76 °C and 584.2 mm at the northern plots near Gould, CO, to 1.51 °C and 425.96 mm at the southern plots near Fraser, CO (HPRCC 2011).

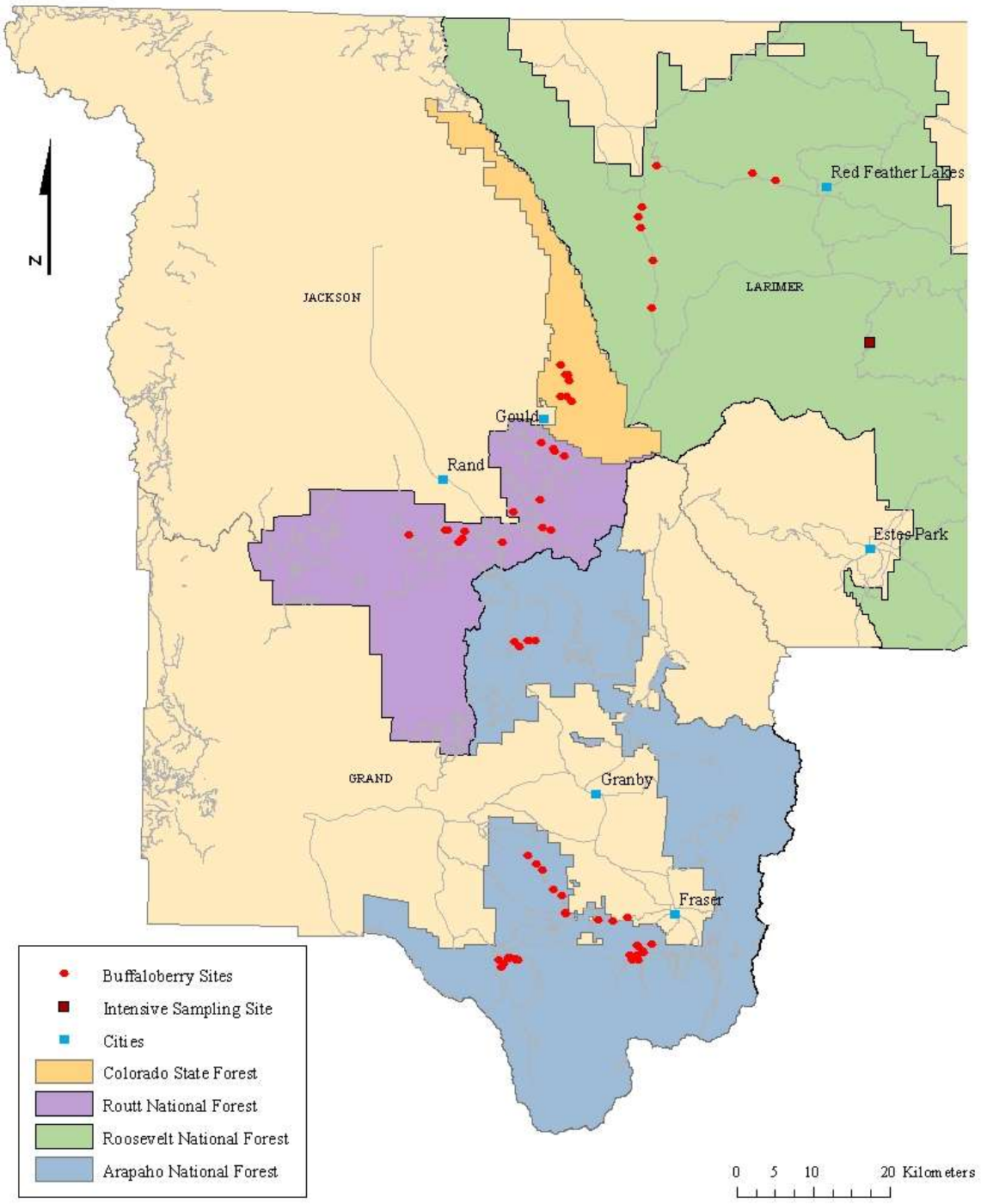

Figure 1. Study plots used to assess N_2-fixation by buffaloberry in Jackson, Larimer, and Grand counties of north central Colorado. Red circles indicate the 59 study plots where foliage from buffaloberry and reference species, soil, and site characteristics were sampled.

Study design and Sampling

Study plots were selected to represent a wide range of buffaloberry cover under various tree canopy densities throughout the study area (Figure A2). Each site was sampled using a 0.1-ha circular plot with a center transect (17.8 m) that ran north (N) to south (S) across the diameter of the plot (Figure 2). Two 12-m transects ran parallel to the center transect, one 4.45 m to the east (E), and one 4.45 m to the west (W) of the center transect. Five sampling points were placed along these three transects. The first point was placed in the center of the plot along the center transect and one point was placed 4.45 m N and S of the center point of each of the E and W transects (Figure 2). Canopy cover was measured at these five points with a meter long LI-COR (Line Quantum Sensor, Lincoln, NE) light sensor to measure the photosynthetically active radiation (PAR) (Figure A1). Four measurements were recorded at each sampling point, one in each of the four cardinal directions, and then averaged. Four PAR measurements were also recorded in full sun up to 20 m from the plot to calculate a relative PAR value for each plot as compared to full sun.

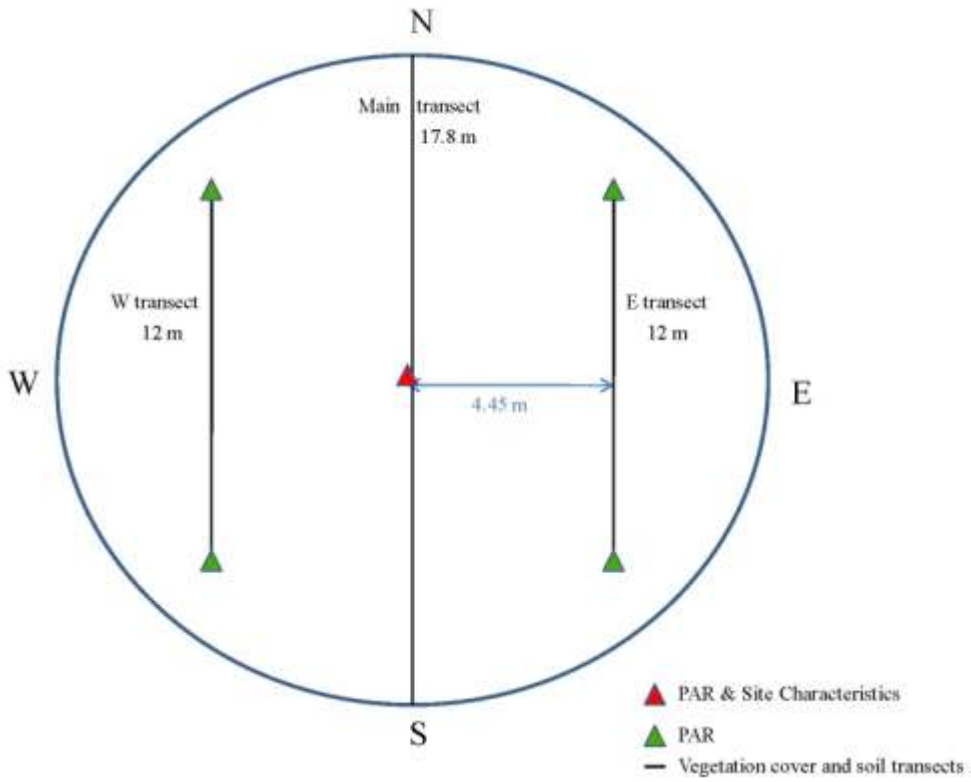

Figure 2. Study plot layout showing three transects used for vegetation cover and soil measurements at 59 sites in north central Colorado, USA. The green triangles indicate four points where photosynthetically active radiation (PAR) was measured. Red triangle indicates the center point where PAR and site characteristics were measured. Buffaloberry foliage was collected throughout the plot whereas reference species foliage was collected at least 2 m from neighboring buffaloberry shrubs either in or outside of the plot.

Tree species composition and basal area were recorded at the center point of each plot by identifying live and dead trees to species, and estimating basal area using a 20 basal area factor glass prism. Other measures recorded at the center point of each plot were slope, aspect, latitude/longitude (GPS), and elevation. Understory species abundance was measured by recording total canopy cover of each shrub species, buffaloberry and reference species, using the line intercept method along the entire length of the three transects (41.8 m).

Foliar samples were collected from up to twelve buffaloberry individuals in each plot. When buffaloberry was very abundant within a plot, foliage was collected in each quarter section

of the plot to obtain a representative sample. Leaves from each shrub were collected at three consistent heights (low, medium, high) at each cardinal direction. Samples were pooled for each plot and no less than a total of 2 g in dry weight was collected of buffaloberry foliage. In addition to foliage, 2 g of litter (the surface portion of the O horizon), mainly composed of pine needles, was collected in each quarter section of the plot at 27 of the study plots (results found in Appendix Table A4).

To determine the N_2-fixing ability of buffaloberry, non-N_2-fixing reference plants were also sampled at each plot. There is a lack of previous studies in Colorado using the ^{15}N natural abundance method to quantify N_2-fixation in lodgepole pine communities. In this insufficiently described system, it is best to sample several reference species to attempt to detect the variability in ^{15}N among non-N_2-fixing species (Shearer and Kohl 1986, Högberg 1997). For this reason, I sampled most co-occurring shrubs found near the plots. Some of those selected had similar rooting patterns to that of buffaloberry, but some did not (USDA 2010). The foliage of five to ten reference shrub individuals was sampled at each site in or near the circular plot. Reference shrubs were a minimum of 2 m upslope or cross-slope from actinorhizal shrubs to reduce chances of sampling shrubs that may have taken-up ^{15}N from neighboring actinorhizal plants. If the sampling crew could not find a reference species 2 m away from a buffaloberry within the plot, we sampled individuals up to 40 m outside the plot. The most common reference shrubs sampled were Wood's rose (*Rosa woodsii* (Lindl.)), kinnikinnick (*Arctostaphylos uva-ursi* (L.) Spreng.), and creeping barberry (*Mahonia repens* (Lindl.) G. Don). Other infrequently found reference shrubs were Rocky Mountain maple (*Acer glabrum* Torr.), fivepetal cliffbrush (*Jamesia americana* Torr. & A. Gray), wax currant (*Ribes cereum* Douglas), whitestem gooseberry (*Ribes inerme* Rydb.), and Scouler's willow (*Salix scouleriana* Barratt ex Hook). The reference shrub foliage was collected using the same aforementioned technique for buffaloberry.

Buffaloberry berries were quantified in each plot by harvesting all berries that occurred within a 1-m x 0.5-m quadrat every meter along the three transects. When berry production was

substantial throughout the plot, sampling intensity was reduced proportionally and the number of quadrats sampled was recorded. The number of berries per square meter was calculated to compare berry production between plots.

Intensive Sampling Site

One of the 59 plots was also used as an intensive sampling site (40° 36' 30 N, 105° 32' 0 W) to detect if there was a difference in foliar ^{15}N and %N at four different dates throughout the growing season in June, July, August, and September (Figure 1). Thirty buffaloberry shrubs were selected within the plot and individually marked. Shrubs of different sizes and ages were selected to get an accurate representation of the population of buffaloberry within the plot. Fifteen reference individuals were also selected and marked to sample concurrently with the buffaloberry. The reference species were selected to avoid neighboring buffaloberry shrubs as described above. The reference species sampled were kinnikinnick, cliffbrush, wax currant, Woods' rose, and willow. Foliage of buffaloberry and reference shrubs was collected using the same techniques described previously. Foliage was not pooled for this plot, rather each individual shrub was analyzed separately using mass spectrometry techniques described below.

Analysis of ^{15}N

Buffaloberry foliage was analyzed with a mass spectrometer and compared to reference shrub foliage using the ^{15}N natural abundance method. The ^{15}N natural abundance method has been used to determine the amount of N derived from N_2-fixation (N_{dfa}) and relies on comparing N_2-fixing and non- N_2-fixing plants to the atmospheric standard of N_2 (0.3663‰) (Shearer and Kohl 1986, Högberg 1997, Boddey et al. 2000, Vitousek et al. 2002). Foliar and litter samples were oven dried at 55° C to a constant mass and ground using a Wiley Mill with a size 20-mesh screen. Samples were weighed and analyzed for ^{15}N content with a Carlo Erba NA 1500

elemental analyzer (C.E. Elantech, Milan Italy) coupled to a VG Isochrom isotope ratio mass spectrometer (Isoprime Inc., Manchester UK). ^{15}N is defined as:

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using atmospheric N_2 as the standard for the ^{15}N:^{14}N ratio ($R_{standard}$). The standard deviation of the mass spectrometer instrument is 0.3‰. Using the ^{15}N natural abundance method, the %N derived from N_2-fixation was calculated using the formula:

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where $\delta^{15}N_{ref}$ is from the reference species, $\delta^{15}N_{fix}$ is from buffaloberry, and B is the $\delta^{15}N$ of the N_2-fixing plant when totally dependent on atmospheric N_2. There are many sources of error in determining B (Högberg 1997, Boddey et al. 2000) and I did not grow buffaloberry in a N-free medium to calculate B. Other studies have used several approaches in using B in the N_{dfa} calculation (Shearer and Kohl 1986, Boddey et al. 2000, Gehring and Vlek 2004, Busse et al. 2007). The typical range for B in woody legumes is −2.0 to +1.0‰ (Boddey et al. 2000). Binkely et al. (1985) grew the actinorhizal red alder in a N-free medium and determined B = − 0.6‰ while Busse et al. (2007) used B = 0‰ for actinorhizal bitterbrush (*Purshia tridentata* (Pursh). I calculated N_{dfa} using B = −1.0‰, − 0.6‰, 0‰, +0.6‰, and +1.0‰ to determine the possible range in N_{dfa} as B changes.

Soil Analyses

Soil samples were collected from the top 10 cm using power drills fitted with a 1.9 cm auger drill bit. Samples were taken in each plot every meter along the three transects, composited per plot, and kept at 4° C for laboratory analysis. Each soil sample was divided into four subsamples. The first and second subsamples were analyzed for net N mineralization and

nitrification rates using an aerobic laboratory incubation (Klute et al. 1994) and the methods are explained in Appendix 1.

The third subsample of each soil sample was sent to AgSource Harris Laboratories in Lincoln, NE and analyzed for total N, total C, P, cations, and buffer pH (results found in Appendix 1). Total N was determined using the Kjedahl digestion method (TKN) (Klute et al. 1994), total C was calculated from the loss of weight on ignition, and P was determined using the Bray I extraction (Klute et al. 1994). Cations (Ca, K, Na, and Mg) were determined using ammonium acetate extraction (Klute et al. 1994). Cation exchange capacity (CEC) was computed by summation after the cation extractions were completed (Klute et al. 1994). Buffer pH was determined using the SMP buffer solution and soluble salts were determined by 1:1 soil/water slurry (Klute et al. 1994). Particle size analysis was completed on each soil sample using the hydrometer measurement (Klute et al. 1994). Lastly, the final subsample was dried to a constant mass, ground on a ball mill, and analyzed for ^{15}N content using the same mass spectrometer as the foliage samples. The results for all soil analyses can be found in Appendix Table A5 and soil classifications by plot can be found in Appendix Table A1. Inorganic N was also measured using ion exchange resin bags at 46 plots (Appendix 3).

Statistical Analyses

Relationships between the vegetation, environmental, and soil variables were examined by inspecting pair-wise correlations computed in SAS PROC Corr (SAS Institute Incorporated, v. 9.2, Cary, NC) (Appendix Table A6). The Dinn-Sidak correction was used to determine α' for the pair-wise correlations ($\alpha' = 0.0034$). The critical *P*-value used in all of the following statistical analyses was $\alpha = 0.05$. A mixed model for response variables foliar $\delta^{15}N$ and % N at all 59 plots was estimated using SAS Proc Mixed with species as the fixed factor and plots as a random factor. The estimation method was restricted maximum likelihood (REML). Post-hoc

comparisons of all reference species to buffaloberry were made using Dunnett's method to identify species differences as a first step in showing active N_2-fixation (Shearer and Kohl 1993). Additionally, a contrast was used to compare the $\delta^{15}N$ of buffaloberry to the average $\delta^{15}N$ of the reference species to estimate the differences in $\delta^{15}N$ values between the N_2-fixing plant and non-N_2-fixing plants. Another contrast was used to compare the %N of buffaloberry to the average %N of the reference species to estimate the difference in foliar N content. Foliar %N was plotted against the $\delta^{15}N$ of each species to illustrate the separation between N_2-fixing buffaloberry and non-N_2-fixing reference plants. Results are reported with *F* statistic, subscript degrees of freedom, and associated *P*-value. The model standard errors of the reference species reflect the low sample size of species (cliffbrush) or that were unreplicated (gooseberry and maple).

Högberg (1997) suggested that there can be some degree of certainty that active biological N fixation is occurring using the N_{dfa} calculation if there is a 5‰ or more difference between $\delta^{15}N_{fix}$ and $\delta^{15}N_{ref}$ ($\delta^{15}N_{DIFF}$). I calculated N_{dfa} using the average of each reference species across all plots, as well as by using the average of all reference species at each plot. Since few plots exhibited the 5‰ difference, I used 13 plots that had at least one reference species with a $\delta^{15}N_{DIFF} > 3.5$‰ to calculate N_{dfa} (Table 5). While using the $\delta^{15}N_{DIFF} > 3.5$‰ may not provide as much certainty in $\%N_{dfa}$ as using a 5‰ difference, it may provide probable evidence of the percentage of N derived from N_2-fixation.

A linear regression was estimated between light availability and N_{dfa} using SAS PROC Reg. Light availability at each plot was compared to N_{dfa} values based on the average of all reference species. This analysis was also conducted using N_{dfa} from the 13 plots with $\delta^{15}N_{DIFF}$ >3.5‰. In addition, average N_{dfa} was calculated separately for kinnikinnick, creeping barberry, and Woods' rose and was compared to light availability, as these species were the only reference species with sufficient samples to use in a regression. N_{dfa} values used in the regression analyses were calculated with B=0‰, since this B had the least amount of variability and one impossible

value (Tables 4 and 5), which was mainly due to the reference species variability, as compared to the other values of B.

At the intensive study site, the response variables, foliar $\delta^{15}N$ and % N, were analyzed using a two-group repeated measures design computed by SAS PROC Mixed (REML estimation). The reference species were combined for this analysis since there were not enough samples of each individual species; therefore the species analyzed were 'buffaloberry' and 'reference species.' In this analysis, the species was the group factor, individual shrubs were nested within groups, with individual shrubs repeatedly sampled over four dates. Preliminary comparisons between species were made from the adjusted least squares means. Due to unequal variance by species, the two-group analysis was considered preliminary and used only to compare species least squares means. Follow-up analyses to compare sampling dates, separately within species, were performed using a single factor (date) repeated measures model.

To determine if there was a relationship between buffaloberry berry production (berries m^{-2}) and relative PAR, an analysis of covariance model was used with relative PAR as a continuous predictor and location was a categorical variable. The plots were separated into 9 location categories for this analysis, since shrubs in different locations were at different stages of berry production. The location categories were separated by distance, terrain, or elevation. Slopes were parallel in the analysis of covariance model. The $\log_e$ was used to stabilize variance to satisfy the homogeneity of variance assumption, and 0.02 was added so that values of zero could be used in the analysis.

RESULTS

The mixed-model estimated mean of the $\delta^{15}N$ of buffaloberry (−0.63‰) across all 59 plots was closer to the atmospheric standard (0.3663‰) as compared to the average of all reference shrubs (−3.27‰) (Table 2). Among species, average estimated mean $\delta^{15}N$ values ranged from −0.63‰ to −4.81‰, with buffaloberry being significantly more enriched in ^{15}N

(Table 2) ($F_{8, 151}$ = 17.00, P <0.0001). On average the $\delta^{15}N$ of buffaloberry was 2.64‰ higher than the reference species (t_{164} = −7.09, P <.0001).

Table 2. Least square mean values of foliar $\delta^{15}N$ (‰), standard errors (SE), number of observations, and 95% confidence intervals (CI) of buffaloberry and the non-N_2-fixing reference shrubs among all sampled plots in north central Colorado. Means, SEs, and CIs were determined using a mixed-model where species was the fixed factor and plots sampled were the random factor.

Species	*n*	$\delta^{15}N$ mean(‰) (SE)	95% CI
Buffaloberry	59	−0.63 (0.19)	−1.02, −0.25
Wood's rose	58	−1.29 (0.20)	−1.68, −0.90
Scouler's Willow	11	−1.50 (0.44)	−2.37, −0.64
Creeping barberry	39	−1.97 (0.24)	−2.44, −1.50
Five petal cliffbrush	2	−3.13 (1.01)	−5.13, −1.14
Kinnikinnick	17	−4.72 (0.35)	−5.42, −4.03
Whitestem gooseberry	1	−4.15 (1.43)	−6.96, −1.33
Wax currant	2	−4.59 (1.01)	−6.60, −2.59
Rocky Mountain maple	1	−4.81 (1.42)	−7.60, −2.00

Species key: Creeping barberry (*Mahonia repens*); Fivepetal cliffbrush (*Jamesia americana*); Kinnikinnick (*Arctostaphylos uva-ursi*); Rocky Mountain Maple (*Acer glabrum*); Russet buffaloberry (*Shepherdia canadensis*); Scouler's willow (*Salix scouleriana*); Wax currant (*Ribes cereum*); Whitestem gooseberry (*Ribes inerme*); Wood's rose (*Rosa woodsii*).

Significant differences in $\delta^{15}N$ were observed between buffaloberry and four of the reference shrubs based on Dunnett's adjusted *P*-values (Figure 3). The standard errors and confidence intervals were based on the mixed-model. Gooseberry was only sampled at one plot and cliffbrush was sampled at two plots; therefore the standard errors were relatively large. Both these species had a fairly large estimated difference in $\delta^{15}N$ that was significant from buffaloberry when taken by itself, but not significant when adjusted for Dunnett's test (Appendix Table A2).

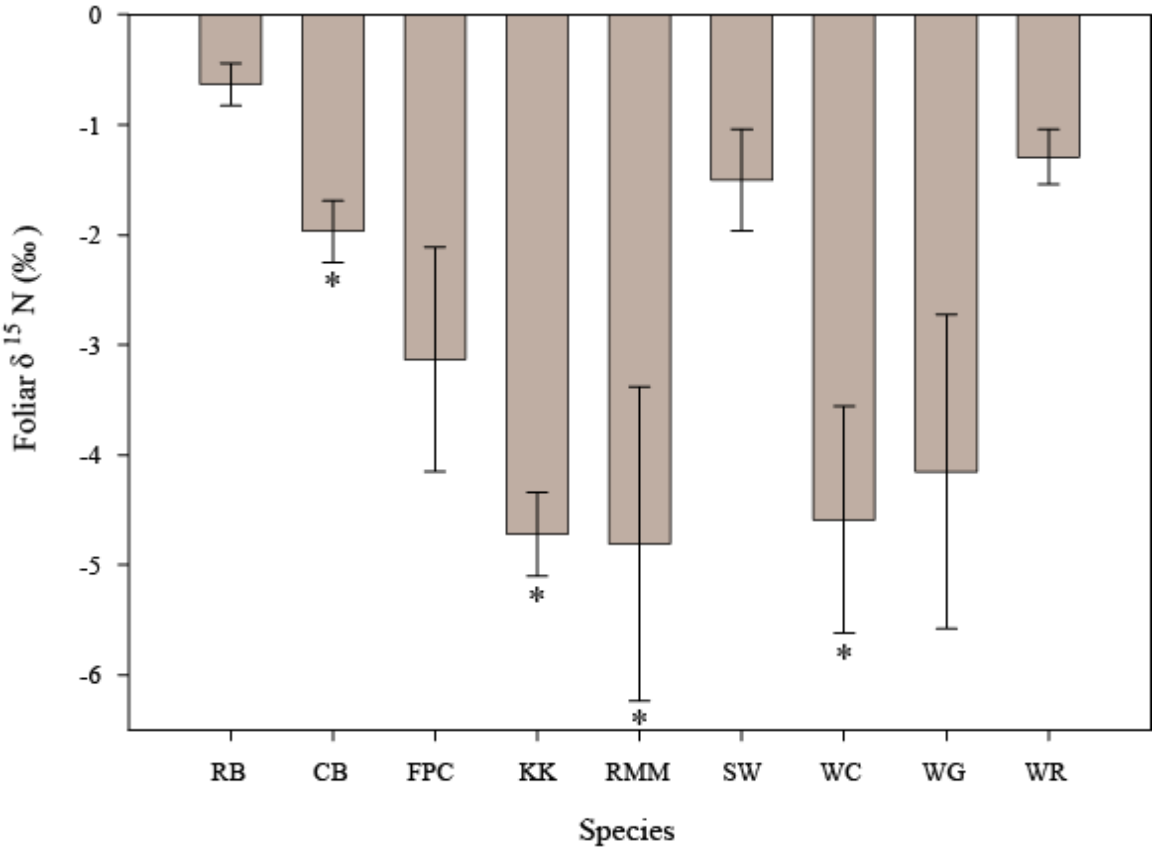

Figure 3. Least square means and standard errors (SE) of the foliar $\delta^{15}N$ of reference species and buffaloberry among all sampled plots in north central Colorado. Means and SEs were determined using a mixed-model where species was the fixed factor and plots sampled were the random factor. * Indicates reference species significantly different from buffaloberry using Dunnett's adjusted *P*-value ($\alpha = 0.05$).
Species key: CB = Creeping barberry (*Mahonia repens*); FPC = Fivepetal cliffbrush (*Jamesia americana*); KK = Kinnikinnick (*Arctostaphylos uva-ursi*); RB = Russet buffaloberry (*Shepherdia canadensis*); RMM = Rocky Mountain Maple (*Acer glabrum*); SW = Scouler's willow (*Salix scouleriana*); WC = Wax currant (*Ribes cereum*); WG = Whitestem gooseberry (*Ribes inerme*); WR = Wood's rose (*Rosa woodsii*).

Foliar %N values ranged from 1.11% to 3.48% with buffaloberry having the highest %N (Table 3). Foliar %N differed significantly by species ($F_{8, 145} = 98.38$, $P < 0.0001$). On average, the foliar %N of buffaloberry was 1.19% ($t_{160} = -12.16$, $P < .0001$) higher than the reference species. Based on the Dunnett's multiple comparison adjustment to the *P*-values there were also significant differences in foliar %N between buffaloberry and five of the individual reference species (Figure 4). Both maple and gooseberry were only sampled at one plot each, they had a fairly large estimated difference in %N that was significant from buffaloberry when taken by

itself, but not significant when adjusted for Dunnett's test (Appendix Table A3). Figure 5 shows foliar $\delta^{15}N$ versus % N of N_2-fixing buffaloberry and non-N_2-fixing species. When taken together, the higher foliar $\delta^{15}N$ and %N of buffaloberry compared to reference species indicates that buffaloberry is fixing considerable N_2.

Table 3. Least square mean values of foliar %N, standard errors (SE), and 95% confidence intervals (CI) of buffaloberry and the non-N_2-fixing reference shrubs among all sampled plots in north central Colorado. Means, SEs, and CIs were determined using a mixed-model where species was the fixed factor and plots sampled were the random factor.

Species	*n*	% N mean (SE)	95% CI
Kinnikinnick	17	1.11 (0.09)	0.93, 1.30
Fivepetal cliffbrush	2	2.22 (0.27)	1.70, 2.74
Creeping barberry	39	2.02 (0.06)	1.90, 2.15
Wax currant	2	2.32 (0.27)	1.79, 2.85
Whitestem gooseberry	1	2.50 (0.37)	1.76, 3.24
Rocky Mountain maple	1	2.51 (0.37)	1.77, 3.24
Wood's rose	58	2.41 (0.05)	2.31, 2.51
Scouler's willow	11	3.20 (0.12)	2.97, 3.43
Russet buffaloberry	59	3.48 (0.05)	3.37, 3.58

See Table 2 for species key.

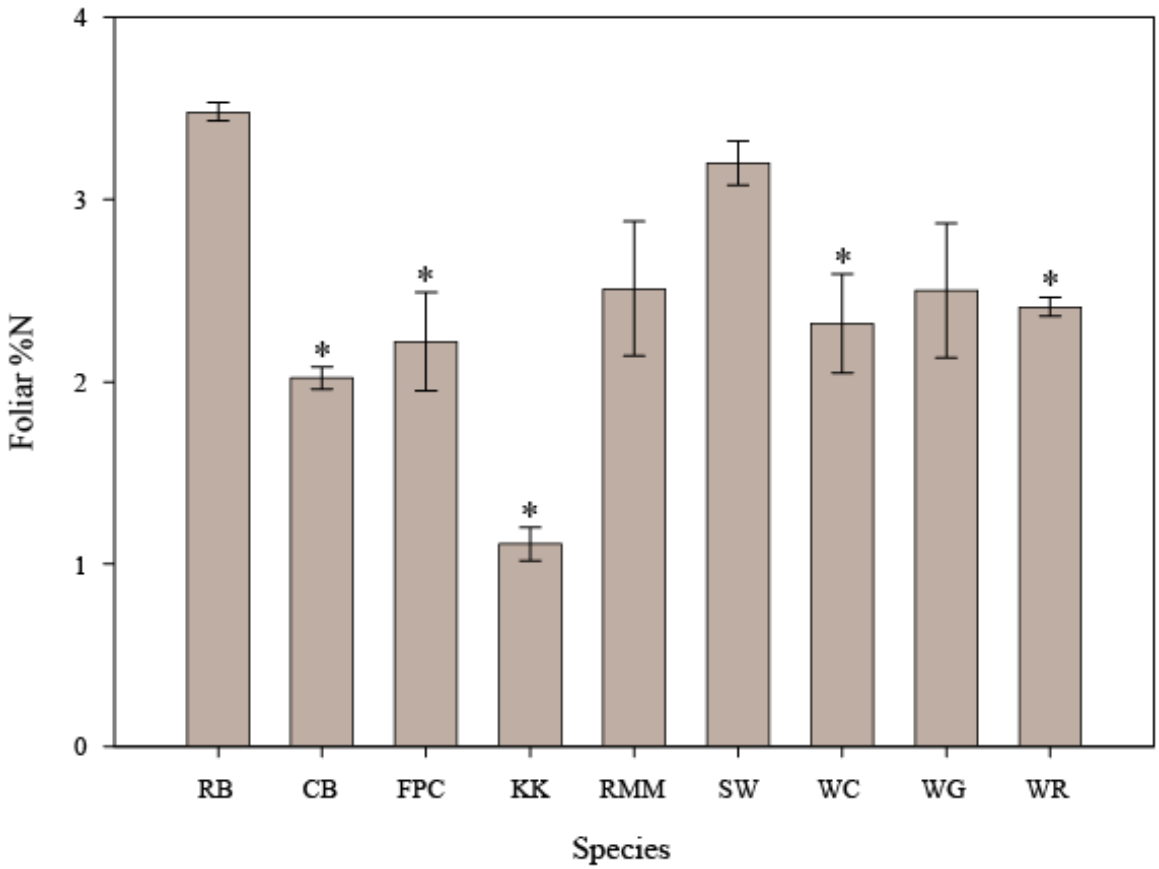

Figure 4. Least square means and standard errors (SE) of the foliar % N of reference species and buffaloberry among all sampled plots in north central Colorado. Means and SEs were determined using a mixed-model where species was the fixed factor and plots sampled were the random factor. * Indicates reference species significantly different from buffaloberry using Dunnett's adjusted *P*-value.

Species key: CB = Creeping barberry (*Mahonia repens*); FPC = Fivepetal cliffbrush (*Jamesia americana*); KK = Kinnikinnick (*Arctostaphylos uva-ursi*); RB = Russet buffaloberry (*Shepherdia canadensis*); RMM = Rocky Mountain Maple (*Acer glabrum*); SW = Scouler's willow (*Salix scouleriana*); WC = Wax currant (*Ribes cereum*); WG = Whitestem gooseberry (*Ribes inerme*); WR = Wood's rose (*Rosa woodsii*).

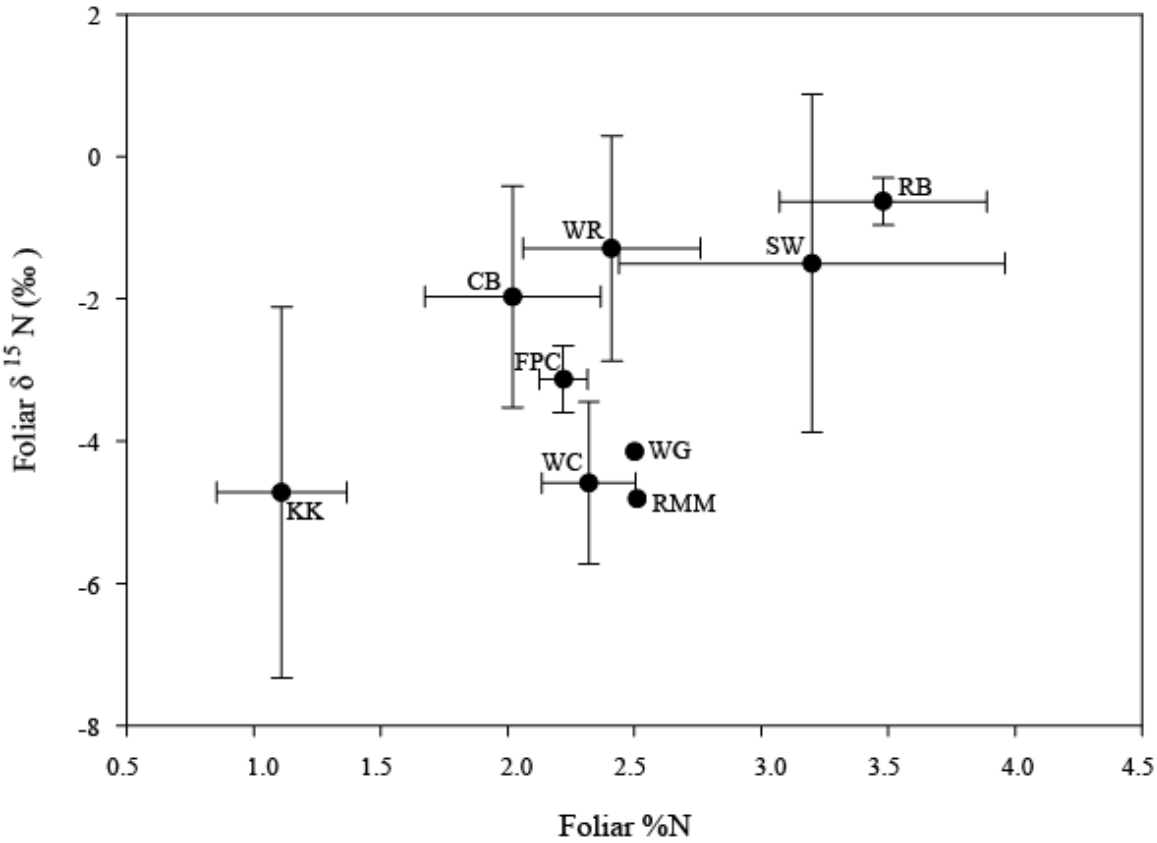

Figure 5. Least square means of foliar $\delta^{15}N$ versus %N, with corresponding standard deviation (SD) bars, of reference species and N_2-fixing buffaloberry across all sampled plots in north central Colorado. Means and SDs were determined using a mixed-model where species was the fixed factor and plots sampled were the random factor. Buffaloberry is clustered slightly below 0‰ with high foliar %N, while the reference species are more variable in $\delta^{15}N$ and tend to have lower %N values. WG and RMM do not have SD bars because the sample size was *n*=1. FPC had a sample size of *n*=2.

Species key: CB = Creeping barberry (*Mahonia repens*); FPC = Fivepetal cliffbrush (*Jamesia americana*); KK = Kinnikinnick (*Arctostaphylos uva-ursi*); RB = Russet buffaloberry (*Shepherdia canadensis*); RMM = Rocky Mountain Maple (*Acer glabrum*); SW = Scouler's willow (*Salix scouleriana*); WC = Wax currant (*Ribes cereum*); WG = Whitestem gooseberry (*Ribes inerme*); WR = Wood's rose (*Rosa woodsii*).

Using different reference species and different values of B altered the N_{dfa} calculation. The average N_{dfa} among all reference species and values of B ranged from 59-86% (Table 4). The $\delta^{15}N$ values of the reference species were highly variable and often unrealistically over- or underestimated N_{dfa}. This was especially the case with willow, as the $\delta^{15}N$ was not uniformly less enriched than the $\delta^{15}N$ of buffaloberry. The $\delta^{15}N_{ref}$ (willow) was often similar to $\delta^{15}N_{fix}$ (buffaloberry); therefore, the N_{dfa} often impossibly exceeded 100%. When N_{dfa} is high, the value

of B can drastically affect the N_{dfa} estimates (Doughton et al. 1992), and this issue can be compounded by highly variable $\delta^{15}N_{ref}$.

Table 4. Estimates of percent nitrogen derived from fixation (N_{dfa}) calculated at all plots (*n*=59) in north central Colorado using foliar $\delta^{15}N_{fix}$ of buffaloberry and foliar $\delta^{15}N_{ref}$ of the listed reference species. The $\delta^{15}N$ of all reference species were averaged at each plot and was also used to calculate N_{dfa}. N_{dfa} was calculated using different values of B to estimate the potential range of N_{dfa} for all reference species. B is the $\delta^{15}N$ of buffaloberry when completely dependent on atmospheric N_2.

	N_{dfa} (%)				
Reference Species	B = −1‰	B = −0.6‰	B = 0‰	B = +0.6‰	B = +1‰
Rocky Mountain maple	>100*	>100*	90	81	>100*
Kinnikinnick	>100*	92	83	84	>100*
Fivepetal cliffbrush	>100*	>100*	82	22	< 0*
Creeping barberry	>100*	>100*	69	6	< 0*
Wax currant	>100*	>100*	83	28	< 0*
Whitestem gooseberry	>100*	>100*	93	83	77
Wood's rose	>100*	>100*	91	30	< 0*
Scouler's willow	>100*	>100*	>100*	>100*	95
All reference species (avg)	>100*	>100*	86	59	63

* Indicates unrealistic values of N_{dfa}.
See Table 2 for species key.

The 13 plots with $\delta^{15}N_{DIFF}$>3.5‰ have more consistent estimates of N_{dfa} since the $\delta^{15}N_{ref}$ are less variable. Depending on the value of B used, the average $\%N_{dfa}$ values averaged over all species with $\delta^{15}N_{DIFF}$>3.5‰ ranged from 76% to 95% (Table 5). Regardless of method used to calculate $\%N_{dfa}$, buffaloberry obtains over half of foliar N from biological N fixation.

Table 5. Estimates of percent nitrogen derived from N_2-fixation (N_{dfa}) calculated at 13 plots with $\delta^{15}N_{ref}$ (reference species) values that were greater than 3.5‰ from $\delta^{15}N_{Fix}$ (buffaloberry). $\delta^{15}N_{DIFF}$ is the absolute value of the difference in $\delta^{15}N$ between buffaloberry and reference species listed. $\%N_{dfa}$ was calculated using different values of B to estimate the potential range of $\%N_{dfa}$. B is the $\delta^{15}N$ of buffaloberry when completely dependent on atmospheric N_2.

Plot	Reference Species (N_{ref})	$\delta^{15}N_{DIFF}$ (‰)	N_{dfa} (%)				
			B = −1‰	B = −0.6‰	B = 0‰	B = +0.6‰	B = +1‰
1	Kinnikinnick	5.51	>100*	94	85	78	74
4	Kinnikinnick	5.85	>100*	>100*	94	86	81
5	Kinnikinnick	5.54	>100*	97	88	80	76
8	Kinnikinnick	5.30	>100*	>100*	95	86	81
9	Kinnikinnick	7.24	>100*	>100*	96	89	84
9	Gooseberry	4.48	>100*	>100*	93	83	77
20	Barberry	4.01	>100*	>100*	90	79	74
29	Maple	4.80	>100*	>100*	90	81	76
29	Kinnikinnick	6.74	>100*	>100*	92	85	81
30	Kinnikinnick	6.46	>100*	98	90	83	79
30	Wax currant	5.07	>100*	98	87	79	75
36	Kinnikinnick	8.50	>100*	100	94	88	84
37	Kinnikinnick	3.88	>100*	>100*	88	78	72
44	Kinnikinnick	3.55	98	87	74	65	60
54	Barberry	3.59	>100*	94	81	72	66
59	Barberry	5.81	>100*	>100*	93	85	80
		Average	>100*	95	89	81	76

* Indicates unrealistic N_{dfa} values that exceed 100%.
See Table 2 for species key.

There were no significant correlations between light availability and N_{dfa}, using PAR (Figure A1) as the measure of light availability and B=0. The regression R^2 values were 0.04 or below for all the different $\delta^{15}N_{ref}$ used in the N_{dfa} calculation (Table 6). Therefore the contribution of N fixation to the N economy of buffaloberry did not appear to relate to overstory shading.

Table 6. Regression R^2, 95% confidence intervals (CI), and *P*-values for models comparing light availability to nitrogen derived from fixation (N_{dfa}) by buffaloberry. Photosynthetically active radiation (PAR) was used as the measure of light availability. The N_{dfa} was calculated with B=0 and $\delta^{15}N_{ref}$ of the reference species listed in the table.

$\delta^{15}N_{ref}$ Species	*n*	R^2	CI	*P*-value
Kinnikinnick	17	0.04	−0.245, 0.547	0.4289
Creeping barberry	39	0.03	−1.979, 5.712	0.3317
Wood's rose	58	0.01	−2.647, 1.148	0.4323
All reference species (plot avg)	59	0.0001	−0.874, 0.938	0.9439
13 Plots (species avg)	13	0.02	−0.197, 0.131	0.6632

See Table 2 for species key.

Foliar N Phenology

The preliminary two-way analysis of variance established the difference in foliar $\delta^{15}N$, as well as foliar %N, between buffaloberry and reference species averaged over all dates sampled (Table 7). For foliar $\delta^{15}N$, the average of reference species was significantly different from buffaloberry ($F_{1, 43.8} = 24$, $P < 0.0001$); dates sampled were significantly different ($F_{3, 124} = 39.37$, $P < 0.0001$); and the interaction between species and date sampled was significant ($F_{3, 124} = 2.78$, $P = 0.0441$). For foliar %N, the average of the reference species was significantly different from buffaloberry ($F_{1, 44.2} = 267$, $P < 0.0001$); dates sampled were significantly different ($F_{3, 125} = 155.29$, $P < 0.0001$); and the interaction between species and date sampled was significant ($F_{3, 125} = 11.88$, $P < 0.0001$). For the entire season, the foliar $\delta^{15}N$ of buffaloberry was 2.56‰ more enriched than the average of the reference shrubs, while %N of buffaloberry was 1.48% higher than average of reference shrubs (Table 7). Since variances between these groups are unequal, the *P*-values in the $\delta^{15}N$ and % N are approximate. Although *P*-values are approximate, they are small enough to use to establish differences between species.

Follow-up comparisons of dates within species were done using single-group repeated analyses because the data was not amenable to variance stabilizing transformations. An analysis of foliar %N in the original scale indicated increasing variance with mean, while analysis in log scale indicated decreasing variance with mean, therefore, rather than use a less interpretable transformation, the two groups (buffaloberry and the average of the reference species) were analyzed separately to compare %N between dates sampled. There were also variance differences in $\delta^{15}N$ between buffaloberry and the average of reference species.

Table 7. Least square means, standard errors (SE), and confidence intervals (CI) of foliar $\delta^{15}N$ (‰) and %N (response variables) of buffaloberry and average of reference species (groups) for the entire season using a two-group repeated measure design. There was an approximate significant difference between the two species in $\delta^{15}N$ and %N because of unequal variances (α = 0.05). Difference in $\delta^{15}N$ and difference in foliar %N between buffaloberry and the average of the reference shrubs were also analyzed to show significant differences.

Variable	Mean (SE)	CI	*P*-value
Buffaloberry $\delta^{15}N$ ‰	−0.80 (0.31)	−1.42, −0.18	
Reference $\delta^{15}N$ ‰	−3.36 (0.42)	−4.21, −2.51	
Buffaloberry – Reference (Diff) $\delta^{15}N$ ‰	2.56 (0.52)	1.51, 3.61	<.0001
Buffaloberry % N	3.42 (0.05)	3.31, 3.52	
Reference % N	1.93 (0.07)	1.78, 2.08	
Buffaloberry – Reference (Diff) % N	1.48 (0.09)	1.29, 1.66	<.0001

Species key: Buffaloberry = *Shepherdia canadensis*.

Foliar $\delta^{15}N$ of buffaloberry changed significantly throughout the growing season, steadily declining each month from June to September ($F_{3, 87}$ = 121.48, P <0.0001) (Figure 6). Average foliar $\delta^{15}N$ of reference species was similar from June to August before dropping significantly in September ($F_{3, 37}$ = 4.86, P = 0.0060) (Figure 6). Foliar %N of both buffaloberry and reference species was significantly affected by the date sampled (buffaloberry: $F_{3, 87}$ = 268.45, P <0.0001; reference species: $F_{3, 37.1}$ = 23.67, P <0.0001). Buffaloberry foliar %N decreased each month throughout the growing season (Figure 7). In the reference species, foliar %N declined each month from June to August, but is similar between August and September (Figure 7). Foliar %N in autumn, just before leaf abscission, was 2.65% for buffaloberry and 1.50% for the average of reference species (Figure 7).

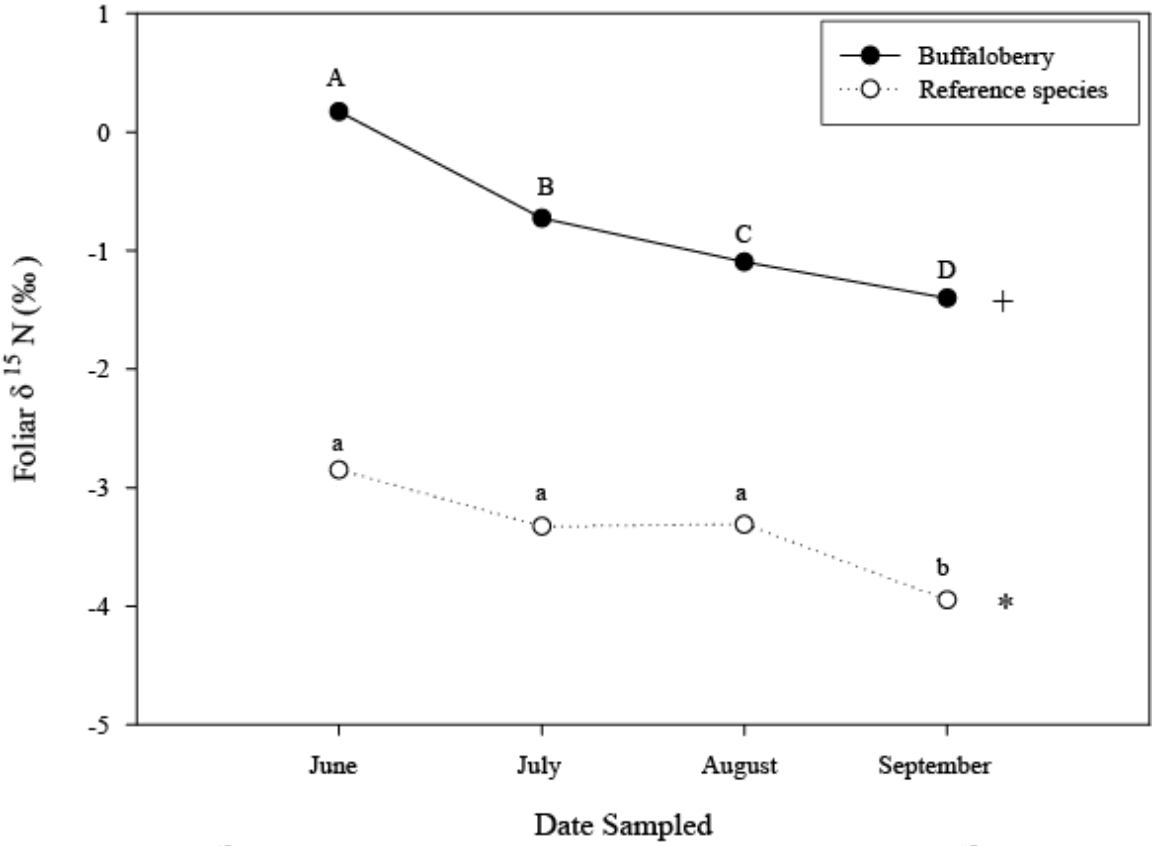

Figure 6. Foliar $\delta^{15}N$ least square means of buffaloberry as compared to foliar $\delta^{15}N$ least square means of the average of reference species throughout the 2009 growing season in north central Colorado. Different letters indicate significant differences in $\delta^{15}N$ between sampling dates for each species using a single factor repeated measures model comparing sampling dates separately within species. ($\alpha = 0.05$). Standard errors (SE) refer to the difference between sampling dates for each species group. Species key: Buffaloberry = *Shepherdia canadensis*.
+SE (diff) = 0.08
*0.26 < SE (diff) < 0.31

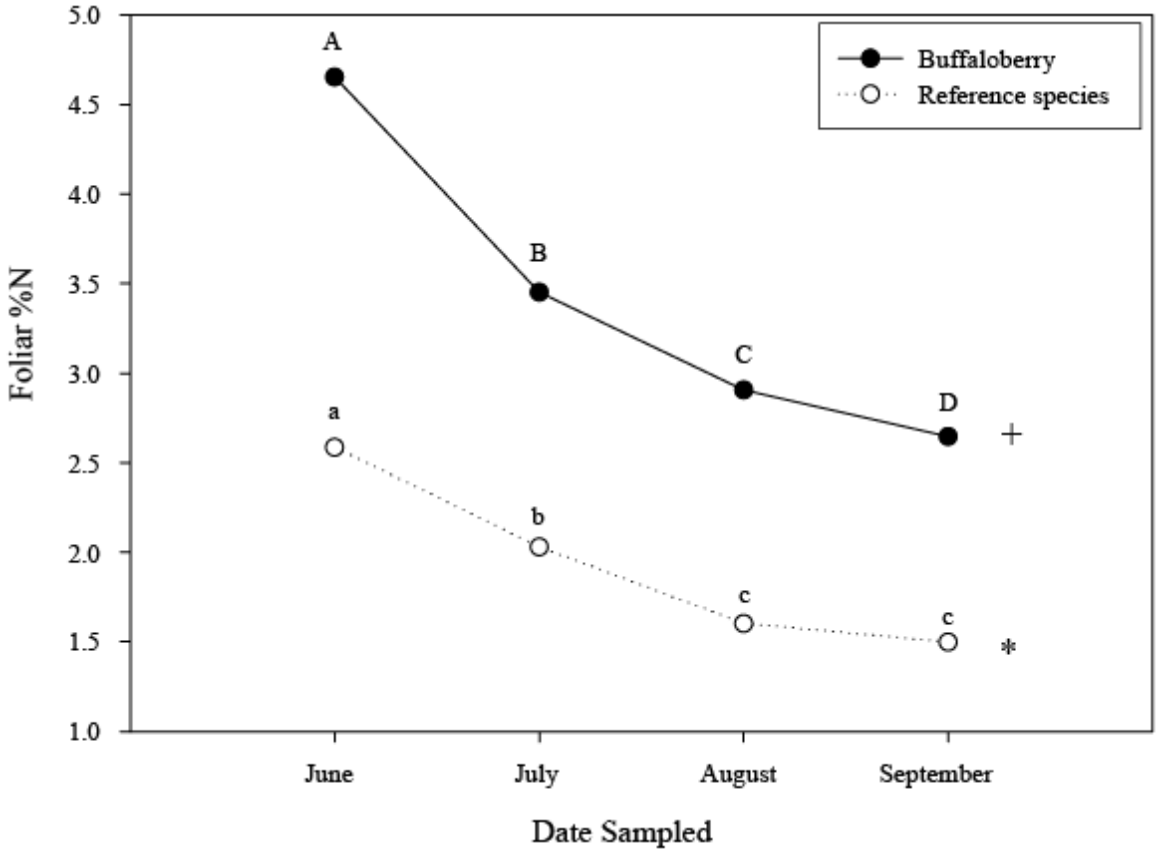

Figure 7. Foliar %N least square means of buffaloberry and foliar %N least square means of the average of reference species throughout the 2009 growing season in north central Colorado. Different letters indicate significant differences in %N between sampling dates for each species using a single factor repeated measures model comparing sampling dates separately within species ($\alpha = 0.05$). Standard errors (SE) refer to the difference between sampling dates for each species group. Species key: Buffaloberry = *Shepherdia canadensis*.
+SE (diff) = 0.08
*0.12 < SE (diff) < 0.15

Berries

The relationship between berry production and light availability was modeled using analysis of covariance with locations as the categorical predictor, and PAR as continuous predictor. Location was significantly different (F_8= 2.57, P = 0.0199) and PAR was significantly different from zero (F_1= 4.45, P = 0.0401) and R^2 = 0.31. The estimated slope with respect to PAR was −0.011 (CI = −0.02, −0.0005). The interpretation of the slope estimate is that a 10% increase in relative PAR is associated with an 11% decrease in berry production, holding location constant.

There were no other environmental, vegetative, or soil variables that were correlated with berry m^{-2} (Appendix A5).

DISCUSSION

Biological N_2-fixation is a difficult process to measure in the field in part because of environmental variation among reference species using the ^{15}N natural abundance approach (Shearer and Kohl 1986, Högberg 1997, Vitousek et al. 2002). In my study area, the results using ^{15}N natural abundance method, along with analysis of foliar N concentrations, suggest that buffaloberry fixes atmospheric N_2 through a symbiotic association with *Frankia* bacteria (Figure 5). The average value of foliar $\delta^{15}N$ of buffaloberry (−0.63‰) is consistent with other studies, which also suggested high amounts of biological N_2-fixation by buffaloberry with similar $\delta^{15}N$ values (−1‰ < ‰ < 0 and −0.4 ‰) close to the atmospheric standard (Kohls et al. 2003, Rhoades et al. 2008). Högberg (1997) suggested that a 5‰ difference between the N_2-fixing shrub ($\delta^{15}N_{fix}$) and the non-fixing shrubs ($\delta^{15}N_{ref}$) is required to provide convincing estimates of $\%N_{dfa}$. However, significant differences in foliar $\delta^{15}N$ have been used in previous studies to suggest N_2-fixation even though the $\delta^{15}N_{DIFF}$ > 5 ‰ was not attained (Shearer and Kohl 1993, Busse et al. 2007, DeCant 2008). There were significant differences in the foliar $\delta^{15}N$ between buffaloberry and the reference species Rocky Mountain maple, kinnikinnick, creeping barberry, and wax currant (Figure 3).

In addition to $\delta^{15}N$ values similar to the atmospheric standard, buffaloberry had a consistently high foliar %N as compared to the non-fixing reference species, again suggesting biological N fixation (Högberg 1986, Vitousek et al. 2002). My study area was a high elevation coniferous forest region that is typically characterized by N-limited soils and low foliar N levels (Rueth and Baron 2002), evidenced by non-fixing plants that had lower foliar %N as compared to buffaloberry (Figure 4). As Högberg (1986) suggests, high foliar N concentration indicates N_2-fixing shrubs use an additional source of N other than what is found in the soil. The foliar $\delta^{15}N$ of

buffaloberry are mostly clustered just below 0‰ with high foliar %N concentrations (Figure 5), implying a reliance on symbiotically fixed atmospheric N_2. The abundance of ^{15}N in the reference species is highly variable and is indicative of several sources of soil N in addition to lower foliar %N (Högberg 1986, Högberg 1997).

Because of the variability in foliar ^{15}N of the reference species (Figure 5), there were several instances when there was not an appreciable difference between $\delta^{15}N_{ref}$ and $\delta^{15}N_{fix}$ and N_{dfa} estimates were inaccurate, as seen in other studies (Gehring and Vlek 2004, Busse et al. 2007). This was most notably seen with the reference species willow in this study. This high variability in foliar willow $\delta^{15}N$ is unexplained, meriting future research, and it may be influenced by other symbiotic associations. I chose several reference species to provide a range of N_{dfa} estimates instead of relying on one reference species (Shearer and Kohl 1986). I also used plots with $\delta^{15}N_{DIFF} > 3.5$ ‰ to ensure interpretable N_{dfa} estimates.

A value for B (the $\delta^{15}N$ when buffaloberry is completely dependent on atmospheric N_2) can be determined by growing the N_2-fixing plants in an N-free media, however, my attempts at growing buffaloberry in the greenhouse failed (Appendix 2). It is also difficult to determine an exact value for B for several reasons (Högberg 1986, Boddey et al. 2000), mainly because different strains of *Frankia* could potentially fractionate N differently and thereby change the $\%N_{dfa}$. I hoped to find a range of $\%N_{dfa}$ of buffaloberry, as Boddey et al. (2000) suggested, using different values of B obtained from the literature, however, this was complicated by the small differences in $\delta^{15}N_{DIFF}$. Among all plots sampled (Table 4), there was the least amount of variability in $\%N_{dfa}$ when B = 0 but when B was −0.6‰ or +0.6 ‰ variability or the frequency of impossible results increased. Among the 13 plots (Table 5) with $\delta^{15}N_{DIFF} > 3.5$‰, B became more enriched in $\delta^{15}N$ as $\%N_{dfa}$ decreased, but when B = −0.6‰ there were impossible N_{dfa} estimates suggesting that B must be greater than −0.6‰. These plots provided a probable range of $\%N_{dfa}$ by buffaloberry with the lowest estimate (when B = +1 ‰) being 60% while the highest was 100% (when B = −0.6‰). In determining N_{dfa} in legumes, the B value can significantly

impact N_{dfa} estimates if the $\%N_{dfa}$ is high (Doughton et al. 1992, Boddey et al. 2000). The range of $\%N_{dfa}$ by buffaloberry is relatively high using all the values of B, indicating the B value could potentially have a significant impact on the actual $\%N_{dfa}$ estimates.

A majority of studies have used the ^{15}N natural abundance method to estimate N_{dfa} in legumes. There are few studies estimating N_{dfa} of actinorhizals in natural systems; and none in Colorado. Therefore, it is difficult to compare N_{dfa} estimates from this study to similar studies. N_2-fixation values can change with different temperatures (Houlton et al. 2008), soil moisture levels (Sprent 1972), and nutrient availability (Vitousek and Field 1999), therefore the dry, cold climate in Colorado with nutrient poor conditions could potentially affect N_2-fixation as compared to other regions. In Alaska, buffaloberry was shown to have ^{15}N abundances very close to 0‰ indicating atmospheric N as a considerable source of N through biological fixation (Kohls et al. 2003, Rhoades et al. 2008). Busse et al. (2007) found the N_{dfa} of actinorhizal bitterbrush to be 46% in northern California while Shearer and Kohl (1986) found the N_{dfa} of the two actinorhizals buckbrush (*Ceanothus cuneatus* (Hook.) Nutt.) and chaparral whitethorn (*Ceanothus leucodermis* Greene) to vary between 36-69% and 45-95%, respectively, depending on B and how many years post-fire they were sampled (Table 1). Buffaloberry appears to have similar or higher $\%N_{dfa}$ values as these other actinorhizals showing that buffaloberry could potentially fix an ecologically significant amount of N in north central Colorado.

There was no observed relationship between light availability and N_2-fixation, as measured by $\%N_{dfa}$ (Table 6). This is counter-intuitive as many models predicting variables that most affect N_2-fixation include light availability as one of the most important factors (Vitousek and Field 1999, Rastetter et al. 2001, Vitousek et al. 2002). Several studies have demonstrated that low light availability negatively impacts N_2-fixation activity in both actinorhizals and legumes (Sprent 1973, Heilman and Stettler 1983, Bormann and Gordon 1984, Khadka and Tatsumi 2006), however most of these studies used the acetylene reduction assay or a controlled experiment to determine N_2-fixation. My field survey found the ^{15}N natural abundance method

difficult due to variable $\delta^{15}N$ values in the reference species, as mentioned above; therefore, perhaps this method did not detect the potential change in N_2-fixation under different light conditions. Also, buffaloberry is known to be somewhat shade tolerant (Uresk and Severson 1998, Walls et al. 2000) and N_2-fixation may not be as dependent on high light levels as other N_2-fixing plants are. Since it is found in a wide range of habitats and successional stages, buffaloberry may be uniquely adapted to a variety of environmental conditions (i.e. low vs. high light conditions) as compared to other actinorhizal plants and therefore it would not be surprising that changes in N_2-fixation were not detected. Finally, this study area is a high elevation forest that has semi-arid moisture conditions during the growing season because most of the annual precipitation is due to winter snowfall. Higher light conditions may result in higher soil temperatures causing an increase in evaporation which could decrease soil moisture and ultimately lower N_2-fixation. Percent soil moisture was significantly correlated to relative PAR (R= −0.4, p=0.0019, n=59; Appendix Table A6), indicating a potential relationship between these variables. Future research could explore other resources, such as soil moisture and P availability, which may suppress N_2-fixation in buffaloberry more than light availability. Research could also determine buffaloberry's persistence and density in a variety of successional stages in these forest systems.

It is possible that since I only sampled and compared the foliar $\delta^{15}N$ of each species, that this method did not detect a change in N_2-fixation under different levels of light whereas measuring whole plant $\delta^{15}N$ may have provided more accurate $\%N_{dfa}$ estimates, as well as changes in fixation. Intra-plant distribution of $\delta^{15}N$ could vary enough between the roots and leaves of both buffaloberry and the reference species to alter the whole plant $\delta^{15}N$ significantly (Shearer and Kohl 1986, Kolb and Evans 2002). The $\delta^{15}N$ can vary from 3-7‰ between the leaves and the roots in plants depending on the ecosystem (Evans 2001). Tjepkema et al. (2000) found a −3.15‰ difference between nodules and leaves in silver buffaloberry (*Shepherdia argentea* (Pursh) Nutt.) and the same pattern of $\delta^{15}N$ depletion in other actinorhizal plants. In

northwest Patagonia, regionally native actinorhizals also demonstrated the same pattern of nodule $\delta^{15}N$ depletion as compared to the leaves (Chaia and Myrold 2010). Evans (2001) suggests that it is difficult to use foliar $\delta^{15}N$ levels to determine where the N source originates because values depend on the form of N absorbed, fractionation of assimilated and reallocated N, as well as other possible microbial associations (i.e. mycorrhizal). Khadka and Tatsumi (2006) found that the difference between shoot $\delta^{15}N$ and root $\delta^{15}N$ increased with shading in several legumes. They also saw that the greatest difference between shoot and root $\delta^{15}N$ was found in their reference species, corn (*Zea mays* L.) (Khadka and Tatsumi 2006). There are two possibilities regarding how these factors could have impacted the results of my survey of buffaloberry. First, if shading impacts N_2-fixation in buffaloberry, the isotopic signature could change more in the roots than the leaves and would not be detected by foliar analysis. Second, the reference species could have substantially different $\delta^{15}N$ in roots versus the shoots or leaves and, therefore, when I determined $\%N_{dfa}$, foliar $\delta^{15}N_{ref}$ could have been more enriched than whole plant $\delta^{15}N$.

The ^{15}N natural abundance method may indicate biological N_2-fixation by buffaloberry; however, there are some additional flaws in the method to consider. I did not look at the mineral soil $\delta^{15}N$ or the soil $\delta^{15}N$ at varying depths to determine soil levels of $\delta^{15}N/\ \delta^{14}N$. If biological N fixation is a significant and historical source of N in these systems, this might explain why the total soil $\delta^{15}N$ (−2.72 ‰; Appendix Table A4) is depleted in ^{15}N as compared to the atmospheric standard. Another factor that may have influenced the similar foliar $\delta^{15}N$ values in the reference species as compared to buffaloberry may have been due to co-occuring actinorhizal species (snowbrush (*Ceanothus velutinus* Douglas ex Hook)), as well as several other potentially N_2-fixing legume species (Nevada pea (*Lathyrus lanszwertii* Kellogg var. *leucanthus* (Rydb.) Dorn), silvery lupine (*Lupinus argenteus* Pursh), and goldenbanner (*Thermopsis divaricarpa* A. Nelson)) that may increase the soil isotopic signature of N_2-fixation. At some plots, these potential N_2-fixing legumes were quite abundant. Finally, reference species with variable foliar $\delta^{15}N$ values indicates various sources of N (Figure 5), especially willow in this study. Variation in $\delta^{15}N$ of

reference species may be explained by different N acquisition strategies and may use more NO_3^- while others use more NH_4^+, altering their isotopic composition (Shearer and Kohl 1986, Boddey et al. 2000, Evans 2001). Buffaloberry and reference species may also be affected by other symbiotic relationships, such as mycorrhizal associations, impacting varying foliar $\delta^{15}N$ values (Boddey et al. 2000, Hobbie et al. 2000).

Intensive Sampling Site

The average %N of the reference species and buffaloberry declined throughout the growing season. It has been well documented that generally most plants reallocate about half of their foliar N prior to leaf abscission (Chapin and Kedrowski 1983, Aerts 1996, Kolb and Evans 2002); however actinorhizal plants tend to shed proportionally more foliar N in abscised leaves than non-N_2-fixing plants (Dawson and Funk 1981, Chapin and Kedrowski 1983, Cote and Dawson 1986, Stewart et al. 2008). In my study, just prior to leaf abscission, the foliar N of buffaloberry was 1.15% higher than the average of the reference shrubs, which is consistent with these previous studies noting that actinorhizal tend to retain proportionally less foliar N than non-fixing shrubs.

The average $\delta^{15}N$ of the reference species and buffaloberry also declined through the growing season, however if fractionation is occurring I would expect to see the lighter isotope removed as foliar %N decreases and the residual N becomes more enriched in ^{15}N. Reallocation of N can discriminate against ^{15}N (Shearer and Kohl 1986, Evans 2001) and differs among different species. As mentioned above, change in N source throughout the season could alter the $\delta^{15}N$ in different plant organs because of fractionation in assimilation and reallocation. In a ^{15}N enrichment study, foliar $\delta^{15}N$ did not decline throughout the growing season, suggesting either no change in N source or a low requirements of N in a pine forest ecosystem (Busse 2000). Conversely Sanginga et al. (1996) found a depletion in foliar $\delta^{15}N$ in legumes in an agricultural

setting where there was more of a demand for N. My results suggest that whole plant $\delta^{15}N$ would provide a better picture of where foliar N is being re-allocated or if the N source varies later in the growing season.

Berries

There was a slight decrease in berry production as light availability increased, after adjusting for the nine location categories. This is not intuitive, since the increase in energetic resources would suggest higher input into sexual reproduction (Pitelka et al. 1980). This contradicts Hamer (1996) who found a negative relationship between buffaloberry fruit production and an increase in forest canopy cover, which described 70% of the variability in fruit production. Hamer selected sampling sites based on bear feeding habitat, which may have included the most productive buffaloberry fruiting sites, possibly impacting results. I chose a sampling time (mid-July) that should have been the height of buffaloberry fruiting. However, due to the wide elevational and geographic range of plots, not all plots contained buffaloberry in fruit at the time of sampling and this phonological difference among sites may have confounded the interpretation of these results. To adjust for varying times in fruit production, clustered plots were given categorical location codes in my analysis; however, I also observed that even within one plot fruit production was not consistent from shrub to shrub. In retrospect, I would re-design sampling methods to re-visit each plot until buffaloberry fruiting occurred. Since there is a fairly modest negative slope (−0.011), there is a possibility this result could have occurred by chance due to a type I error or such a result could be observed 5% of the time. My result does not imply causation, and perhaps, some unmeasured variable was not accounted for. In the correlation analysis, berry production was not significantly correlated with any other measured environmental variable such as aspect or elevation (Appendix Table A6). While berry production was not correlated to % soil moisture, there could be an unmeasured interaction between these

two variables. As previously speculated, higher light availability could decrease soil moisture by evaporation, and could negatively impact N_2-fixation and fruit production. Further research is needed to investigate this relationship.

Management Implications

My results suggest that buffaloberry is deriving a significant proportion of N via biological fixation. This is demonstrated by a 60% or greater percent of N derived from N_2-fixation and high foliar %N concentrations in buffaloberry compared to reference species. N-enriched buffaloberry foliage and roots may contribute a significant portion of new N to the system either through senescence or herbivory. As a potentially important source of N in a variety of successional habitats in these N-limited high elevation pine forests, buffaloberry could be a tool for managers by aiding in the recovery of disturbed lands. $\%N_{dfa}$ estimates provided by this study could be coupled with buffaloberry production to determine the amount of N buffaloberry adds to these systems annually.

Since buffaloberry is a shade-tolerant species and found in a variety of successional habitats, I speculate that N_2-fixation by buffaloberry may not be greatly affected by light availability. As the Colorado forests change in response to the mountain pine beetle, managers may expect there to be little impact on N_2-fixation in buffaloberry.

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APPENDIX 1

Site Characteristics

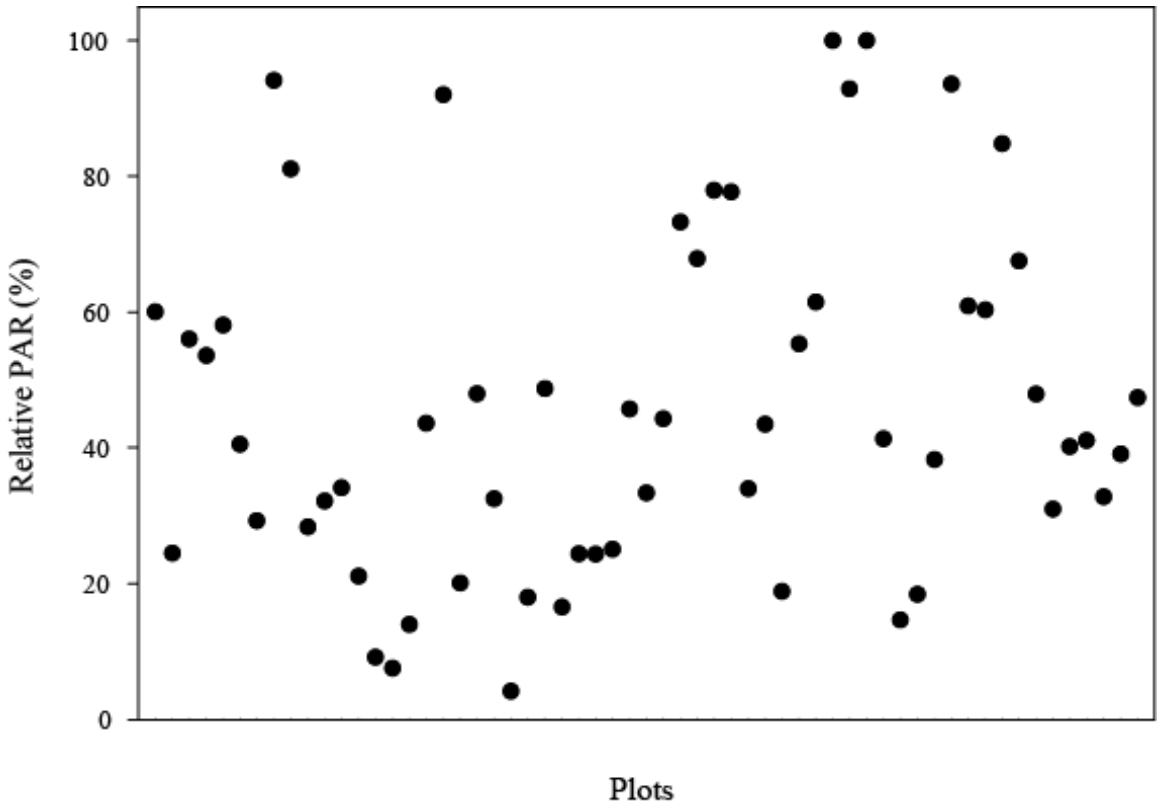

Figure A1. Relative photosynthetic active radiation (PAR) across the 59 plots in north central Colorado comparing N derived from fixation by buffaloberry under different amounts of light availability.

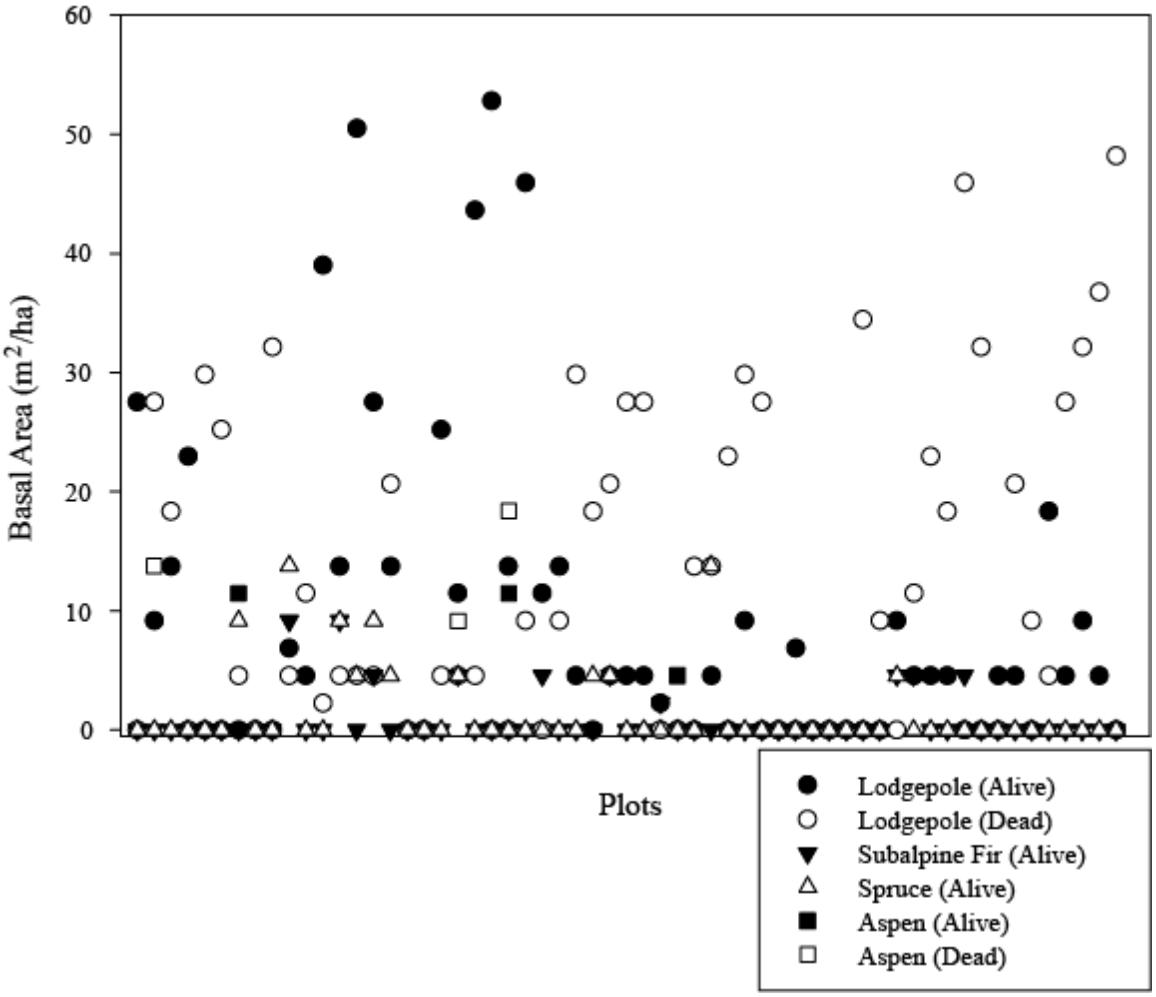

Figure A2. Distribution of the basal area (BA) by species across 59 study plots in north central Colorado. Species: Lodgepole = *Pinus contorta*; Subalpine fir = *Abies lasiocarpa*; Spruce = *Picea engelmannii*; Aspen = *Populus tremuloides*.

Table A1. Soil classification by plot obtained from Natural Resource Conservation Service Soil Web Survey (Date Accessed May 2, 2011).

Plot	Soil Classification
1	Loamy-skeletal, mixed, superactive ustic haplocryalfs; loamy-skeletal, micaceous, shallow ustic dystricryepts
2	no data
3	no data
4	no data
5	no data
6	no data
8	no data
9	no data
10	no data
12	no data
13	no data
14	no data
15	Fine, smectitic ustic argicryolls
16	Fine, smectitic eutric glossocryalfs
17	Loamy-skeletal, mixed, superactive ustic glossocryalfs
19	Loamy-skeletal, mixed, superactive lamellic haplocryalfs
20	Loamy-skeletal, mixed, superactive ustic haplocryalfs
21	Loamy-skeletal, mixed, superactive ustic haplocryalfs
22	no data
23	Loamy-skeletal, mixed, superactive typic dystrocryepts
24	Loamy-skeletal, mixed, superactive lamellic haplocryalfs
25	Loamy-skeletal, mixed, superactive lamellic haplocryalfs
26	Loamy-skeletal, mixed, superactive typic dystrocryepts
28	Loamy-skeletal, mixed, superactive typic dystrocryepts
29	Loamy-skeletal, mixed, superactive ustic haplocryalfs; Loamy-skeletal, micaceous, shallow ustic dystricryepts
30	Loamy-skeletal, micaceous lamellic haplocryepts; Loamy-skeletal, micaceous, shallow ustic dystricryepts
31	no data
32	Loamy-skeletal, mixed, superactive typic dystrocryepts
33	Loamy-skeletal, mixed, superactive ustic haplocryalfs; Loamy-skeletal, micaceous, shallow ustic dystricryepts
34	Loamy-skeletal, mixed, superactive typic dystrocryepts
35	Loamy-skeletal, mixed, superactive typic dystrocryepts
36	Loamy-skeletal, mixed, superactive typic dystrocryepts
37	Loamy-skeletal, mixed, superactive ustic haplocryalfs; Loamy-skeletal, micaceous, shallow ustic dystricryepts
38	Loamy-skeletal, mixed, superactive ustic haplocryalfs; Loamy-skeletal, micaceous, shallow ustic dystricryepts
39	Fine, smectitic vertic haplocryalfs; Loamy-skeletal, mixed, superactive ustic haplocryalfs;
40	Fine, smectitic vertic haplocryalfs; Loamy-skeletal, mixed, superactive ustic haplocryalfs;
42	Sandy, skeletal, mixed, lamellic haplocryepts
43	Sandy, skeletal, mixed, lamellic haplocryepts
44	Sandy, skeletal, mixed, lamellic haplocryepts
45	Sandy, skeletal, mixed, lamellic haplocryepts
46	Sandy, skeletal, mixed, lamellic haplocryepts
47	Sandy, skeletal, mixed, lamellic haplocryepts
48	Sandy, skeletal, mixed, lamellic haplocryepts
50	Sandy, skeletal, mixed, lamellic haplocryepts

Plot	Soil Classification
51	Loamy-skeletal, mixed, superactive eutric haplocryalfs; Fine-loamy, mixed, superactive ustic glossocryalfs
53	Loamy-skeletal, mixed, superactive ustic haplocryalfs; Fine-loamy, mixed, superactive ustic haplocryalfs; Loamy-skeletal, mixed, superactive ustic haplocryepts
54	Loamy-skeletal, mixed, superactive eutric haplocryalfs; Fine-loamy, mixed, superactive ustic glossocryalfs
55	Loamy-skeletal, mixed, superactive eutric haplocryalfs; Fine-loamy, mixed, superactive ustic glossocryalfs
57	Loamy-skeletal, mixed, superactive ustic haplocryalfs; Fine-loamy, mixed, superactive ustic haplocryalfs; Loamy-skeletal, mixed, superactive ustic haplocryepts
58	Loamy-skeletal, mixed, superactive ustic haplocryalfs; Fine-loamy, mixed, superactive ustic haplocryalfs; Loamy-skeletal, mixed, superactive ustic haplocryepts
59	Layey-skeletal, smectitic ustic argicryolls
60	Loamy-skeletal, mixed, superactive ustic haplocryalfs; Fine-loamy, mixed, superactive ustic haplocryalfs; Loamy-skeletal, mixed, superactive ustic haplocryepts
61	Loamy-skeletal, mixed, superactive ustic haplocryalfs; Fine-loamy, mixed, superactive ustic haplocryalfs; Loamy-skeletal, mixed, superactive ustic haplocryepts
62	Loamy-skeletal, mixed, superactive ustic haplocryalfs; Fine-loamy, mixed, superactive ustic haplocryalfs; Loamy-skeletal, mixed, superactive ustic haplocryepts
63	Loamy-skeletal, mixed, superactive ustic haplocryalfs; Fine-loamy, mixed, superactive ustic haplocryalfs; Loamy-skeletal, mixed, superactive ustic haplocryepts
64	Loamy-skeletal, mixed, superactive ustic haplocryalfs; Fine-loamy, mixed, superactive ustic haplocryalfs; Loamy-skeletal, mixed, superactive ustic haplocryepts
65	Loamy-skeletal, mixed, superactive ustic haplocryalfs; Fine-loamy, mixed, superactive ustic haplocryalfs; Loamy-skeletal, mixed, superactive ustic haplocryepts
66	Loamy-skeletal, mixed, superactive ustic haplocryalfs; Fine-loamy, mixed, superactive ustic haplocryalfs; Loamy-skeletal, mixed, superactive ustic haplocryepts

Additional Methods

Soil samples were collected from the top 10 cm using power drills fitted with a 1.9 cm auger drill bit. Samples were taken in each plot every meter along the three transects, composited per plot, and kept at 4° C for laboratory analysis. Each soil sample was divided into four subsamples. The first and second subsamples were analyzed for net N mineralization and nitrification rates using an aerobic laboratory incubation (Klute et al. 1994). The incubation involved extracting a portion (1 g) of each soil sample with 2 M KCl and filtering before analyzing by spectrophotometry for NO_3^- and NH_4^+ on an Alpkem Flow Solution IV automated wet chemistry system (Perstorp Analytical, College Station, Texas) to obtain an initial measurement of inorganic N. The second subsample was incubated for 28 days at 25° C while maintaining the initial soil moisture content throughout the incubation. After incubation, the

subsample was extracted and analyzed for NO_3^- and NH_4^+. Potential net mineralization was calculated by subtracting the initial soil inorganic N from the post-incubation soil inorganic N. Net nitrification was also calculated by dividing the change in NO_3^- by the incubation time. Methods involving the third and fourth subsamples are explained in the main text.

Additional Results

Table A2. Differences in foliar $\delta^{15}N$ (‰) between buffaloberry and reference species individually compared (*P*-value) and using a post-hoc adjustment (Dunnett's *P*-value) among all sampled plots ($\alpha = 0.05$). $\delta^{15}N_{DIFF}$ least square means and SEs were determined using a mixed-model where species was the fixed factor and plots sampled were the random factor.

Species	$\delta^{15}N_{DIFF}$ mean (SE) (‰)	*P*-value	Dunnett's *P*-value
Creeping barberry	−1.34 (0.28)	<.0001*	<.0001*
Five petal cliffbrush	−2.50 (1.02)	0.0153*	0.1122
Kinnikinnick	−4.09 (0.38)	<.0001*	<.0001*
Rocky Mountain maple	−4.17 (1.43)	0.0040*	0.0308*
Scouler's Willow	−0.87 (0.46)	0.0613	0.3806
Wax currant	−3.96 (1.03)	0.0002*	<.0013*
Whitestem gooseberry	−3.51 (1.43)	0.0153*	0.1125
Wood's rose	−0.66 (0.25)	0.0088*	0.0650

*Indicates significance

Species key: Creeping barberry (*Mahonia repens*); Fivepetal cliffbrush (*Jamesia americana*); Kinnikinnick (*Arctostaphylos uva-ursi*); Rocky Mountain Maple (*Acer glabrum*); Russet buffaloberry (*Shepherdia canadensis*); Scouler's willow (*Salix scouleriana*); Wax currant (*Ribes cereum*); Whitestem gooseberry (*Ribes inerme*); Wood's rose (*Rosa woodsii*).

Table A3. Differences in the foliar N (%) between buffaloberry and reference species individually compared (*P*-value) and using a post-hoc adjustment (Dunnett's *P*-value) among all sampled plots in north central Colorado ($\alpha = 0.05$). $\%N_{DIFF}$ least square means and SEs were determined using a mixed-model where species was the fixed factor and plots sampled were the random factor.

Species	$\%N_{DIFF}$ mean (SE)	*P*-value	Dunnett's *P*-value
Creeping barberry	−1.45 (0.07)	<.0001*	<.0001*
Five petal cliffbrush	−1.26 (0.27)	<.0001*	<.0001*
Kinnikinnick	−2.36 (0.10)	<.0001*	<.0001*
Rocky Mountain maple	−0.97 (0.37)	0.0107*	0.0803
Scouler's Willow	−0.28 (0.12)	0.0232*	0.1653
Wax currant	−1.16 (0.27)	<.0001*	0.0003*
Whitestem gooseberry	−0.98 (0.38)	0.0103*	0.0774
Wood's rose	−1.07 (0.06)	<.0001*	<.0001*

*Indicates significance

See Table A2 for species key.

Table A4. Mean values, standard error (SE), sample size (*n*), and 95% confidence intervals of litter* and total soil $\delta^{15}N$ and %N in some of the plots in north central Colorado.

Species	*n*	Mean (SE)	95% CI
Total Soil $\delta^{15}N$ (‰)	58	−2.72 (0.274)	−3.27, −2.17
Total Soil %N	58	0.169 (0.009)	0.15, 0.19
Litter* $\delta^{15}N$ (‰)	27	−7.49 (0.395)	−8.23, −6.68
Litter* %N	27	1.19 (0.062)	0.99, 1.25

*Litter refers to the surface portion of the O horizon.

Table A5. Results of soil analyses among all sampled plots in north central Colorado.

Plot	Soil $\delta^{15}N$ (‰)	Soil % N	% Soil Moisture	Potential NO_3 (ppm)	Potential NH_4 (ppm)	Total Potential Nitrification (ppm)	Mineral NO_3 (ppm)	Mineral NH_4 (ppm)
1	-4.94	0.162	10.29	0.064	0.046	0.110	-0.073	0.021
2	-3.22	0.198	32.10	0.114	0.840	0.954	-0.099	0.139
3	-2.92	0.093	17.13	0.181	0.024	0.205	-0.128	0.102
4	-3.00	0.101	11.45	0.212	0.070	0.282	-0.117	-0.010
5	-4.67	0.150	22.36	0.242	0.064	0.306	-0.056	0.015
6	-7.67	0.110	22.77	0.046	-0.013	0.033	-0.040	0.046
8	-1.66	0.132	22.68	-0.018	-0.091	-0.108	0.110	0.135
9	-2.00	0.099	15.41	0.619	0.093	0.712	-0.008	0.017
10	-2.10	0.144	32.20	0.007	0.417	0.424	0.102	0.748
12	2.04	0.432	39.38	0.036	0.624	0.660	-0.008	0.188
13	-1.22	0.127	29.41	0.185	-0.212	-0.027	0.099	0.410
14	-6.36	0.181	26.69	-0.001	0.078	0.077	0.028	0.025
15	-3.55	0.169	30.88	-0.096	-0.012	-0.108	0.095	0.045
16	-5.03	0.150	20.55	-0.082	0.008	-0.075	0.136	0.086
17	-2.03	0.224	30.53	0.037	0.323	0.360	0.003	0.167
19	-5.47	0.294	37.96	0.061	0.010	0.071	0.011	0.111
20	-1.65	0.329	38.96	-0.026	-0.408	-0.435	0.021	0.495
21	0.53	0.176	26.26	-0.042	-0.665	-0.707	0.061	0.722
22	-0.78	0.090	17.04	0.056	0.058	0.114	0.036	0.006
23	-0.63	0.144	21.56	0.018	0.094	0.113	0.027	0.017
24	0.00	0.085	11.58	-0.007	0.100	0.093	0.024	0.023
25	-2.38	0.091	14.98	0.028	0.134	0.162	-0.005	0.011
26	0.72	0.208	13.09	0.085	0.189	0.274	-0.053	0.135
28	-4.15	0.095	8.54	0.150	0.146	0.296	-0.079	-0.017
29	-0.56	0.267	17.88	0.187	0.078	0.265	-0.076	0.102

Table A5. Continued

Plot	Total Mineral N (ppm)	Total % C	Bray P (ppm)	% TKN*	K (ppm)	Mg (ppm)	Ca (ppm)	pH	Buffer pH	Soluble Salts
1	-0.052	3.31	32.425	0.1625	111	82	645.5	6.05	7.2	0.125
2	0.041	4.00	35.53	0.172	198	124	1358	5.6	6.9	0.18
3	-0.026	1.80	35.85	0.093	122	91	741	5.9	7	0.14
4	-0.128	1.86	15.94	0.09	76	85	882	5.6	7	0.14
5	-0.040	3.19	14.92	0.117	108	84	715	5.3	6.8	0.14
6	0.006	2.44	27.44	0.083	82	63	476	4.8	6.7	0.14
8	0.245	2.78	19.88	0.14	132	91	1020	5.6	7.1	0.15
9	0.009	1.97	37.01	0.123	137	120	929	5.2	6.7	0.18
10	0.850	3.74	67.725	0.1475	232	127	893	5.6	6.8	0.145
12	0.181	8.12	14.27	0.341	354	520	4395	5.3	6.2	0.46
13	0.509	3.02	30.28	0.136	226	207	1544	6	6.8	0.2
14	0.054	4.64	16.02	0.18	132	83	721	5.1	6.3	0.2
15	0.140	4.12	26.43	0.149	73	190	1396	5.5	6.9	0.18
16	0.223	2.96	33.23	0.129	144	90	655	5	6.5	0.17
17	0.170	4.76	49.16	0.226	164	110	1210	4.9	6.6	0.2
19	0.122	7.60	29.32	0.289	164	94	656	5	6.3	0.2
20	0.516	8.24	37.56	0.361	221	152	1531	5.5	6.5	0.25
21	0.784	3.77	56.81	0.181	262	178	1688	5.3	6.5	0.26
22	0.042	2.32	15.08	0.09	124	78	596	5.6	6.8	0.14
23	0.044	3.31	39.96	0.153	213	212	1384	5.5	6.6	0.21
24	0.047	2.67	59.67	0.109	213	120	740	5.4	6.9	0.15
25	0.006	2.44	64.62	0.08	203	98	560	5	6.5	0.17
26	0.083	3.60	29.18	0.21	195	109	1379	5.6	7.1	0.16
28	-0.096	2.32	17.12	0.105	123	82	617	5.6	7.1	0.12
29	0.025	6.55	25.58	0.257	145	115	1128	5.7	6.8	0.16

Table A5. Continued

Plot	Na (ppm)	CEC†	% Sand	% Silt	% Clay	Texture
1	11.5	5.45	67.2	24.4	8.4	Sandy Loam
2	13	10.8	47.2	44.4	8.4	Loam
3	10	6.8	79.2	14.4	6.4	Loamy Sand
4	10	7.4	65.2	28.4	6.4	Sandy Loam
5	11	7.4	59.2	30.4	10.4	Sandy Loam
6	9	7.2	61.2	32.4	6.4	Sandy Loam
8	8	7.8	67.2	28.4	4.4	Sandy Loam
9	9	10	65.2	26.4	8.4	Sandy Loam
10	7.5	8.95	39.2	45.2	15.6	Loam
12	24	36.3	47.2	16.4	36.4	Sandy Clay
13	11	12.9	21.2	60.4	18.4	Silt Loam
14	9	12.7	49.2	36.4	14.4	Loam
15	20	11.2	43.2	48.4	8.4	Loam
16	10	10.4	61.2	28.4	10.4	Sandy Loam
17	16	12.5	29.2	60.4	10.4	Silt Loam
19	11	12.5	63.2	28.4	8.4	Sandy Loam
20	14	15.5	43.2	44.4	12.4	Loam
21	15	16.7	35.2	44.4	20.4	Loam
22	12	6.8	53.2	38.4	8.4	Sandy Loam
23	11	14.3	67.2	22.4	10.4	Sandy Loam
24	12	7.7	63.2	32.4	4.4	Sandy Loam
25	13	10.2	51.2	38.4	10.4	Loam
26	11	10	63.2	34.4	2.4	Sandy Loam
28	11	5.7	65.2	12.4	22.4	Sandy Clay Loam
29	8	9.8	53.2	34	12.8	Sandy Loam

Table A5. Continued

Plot	Soil $\delta^{15}N$ (‰)	Soil % N	% Soil Moisture	Potential NO_3 (ppm)	Potential NH_4 (ppm)	Total Potential Nitrification (ppm)	Mineral NO_3 (ppm)	Mineral NH_4 (ppm)
30	-2.90	0.119	13.87	0.168	0.686	0.854	-0.074	0.062
31	.	.	19.04	0.073	-0.026	0.047	-0.048	0.102
32	-4.22	0.249	26.75	0.200	0.295	0.495	-0.131	0.034
33	-6.80	0.157	16.40	0.204	0.173	0.377	-0.067	0.036
34	-4.73	0.145	19.82	0.146	0.180	0.326	-0.007	0.348
35	-1.37	0.160	20.70	0.078	-0.055	0.024	-0.053	0.240
36	-2.03	0.191	8.09	0.087	0.042	0.129	-0.066	0.113
37	-1.05	0.202	15.70	0.057	0.538	0.595	-0.058	0.354
38	-1.49	0.130	14.57	0.030	0.254	0.284	-0.060	0.068
39	0.19	0.138	12.70	-0.029	0.435	0.406	-0.008	0.106
40	-2.69	0.100	16.25	0.330	0.313	0.643	-0.031	0.322
42	-1.67	0.107	13.48	0.007	0.164	0.171	0.024	0.129
43	-3.52	0.142	16.01	0.056	0.393	0.450	0.018	0.116
44	-2.21	0.151	9.00	0.060	0.174	0.235	0.029	0.123
45	-8.56	0.113	15.10	0.110	-0.023	0.086	-0.025	0.298
46	-2.82	0.098	10.60	0.099	0.050	0.149	-0.012	0.216
47	-5.15	0.189	6.70	0.124	0.101	0.224	-0.013	0.085
48	-2.42	0.223	11.29	0.741	-0.034	0.707	0.095	0.588
50	-2.29	0.239	23.18	-0.031	0.108	0.077	0.088	0.540
51	-2.73	0.212	20.52	0.018	-0.338	-0.320	-0.007	0.808
52	-1.15	0.349	24.14	0.066	0.209	0.274	-0.036	0.135
53	-0.79	0.143	18.03	0.067	0.220	0.287	-0.028	0.082
54	-2.54	0.148	10.25	0.167	0.159	0.326	-0.026	0.090
55	-4.70	0.135	16.83	0.144	0.332	0.476	-0.008	0.183
57	-1.57	0.222	17.09	0.134	0.222	0.357	-0.033	0.114

Table A5. Continued

Plot	Total Mineral N (ppm)	Total % C	Bray P (ppm)	% TKN	K (ppm)	Mg (ppm)	Ca (ppm)	pH	Buffer pH	Soluble Salts
30	-0.013	2.15	41.31	0.101	192	143	1034	5.8	6.8	0.15
31	0.054	1.57	78.12	0.061	163	89	733	5.5	6.7	0.14
32	-0.097	5.86	22.9	0.18	180	115	953	5.2	5.9	0.24
33	-0.031	3.60	27.71	0.117	131	96	823	5	6.5	0.17
34	0.340	2.84	25.46	0.102	202	142	1068	5.1	6.3	0.22
35	0.187	3.65	32.03	0.147	232	191	1603	5.4	6.6	0.21
36	0.047	3.19	6.39	0.124	195	173	1435	5.1	6.7	0.2
37	0.296	4.41	34.73	0.167	247	207	1693	5	6.4	0.24
38	0.008	2.84	18.07	0.116	169	142	1132	5.1	6.6	0.18
39	0.098	2.15	33.12	0.112	204	194	1350	5.3	6.6	0.2
40	0.291	2.49	33.54	0.121	180	164	1142	4.9	6.5	0.19
42	0.152	2.32	10.65	0.094	214	139	931	5	6.7	0.16
43	0.134	3.60	41.24	0.125	226	157	1362	5.1	6.4	0.21
44	0.152	2.44	85.1	0.17	183	161	1482	5.1	6.7	0.18
45	0.273	2.78	34.43	0.103	208	148	1175	5.3	6.7	0.17
46	0.203	2.03	18.8	0.086	178	114	895	5.2	6.5	0.16
47	0.072	2.38	26.11	0.095	148	123	961	4.9	6.6	0.17
48	0.683	3.77	21.77	0.136	222	164	1209	5.3	6.7	0.18
50	0.628	4.52	27.42	0.19	224	150	1321	5.4	6.5	0.23
51	0.801	4.06	80.43	0.217	289	185	2428	5.6	6.8	0.22
52	0.099	9.74	29.71	0.326	128	101	1158	4.7	6.5	0.18
53	0.054	4.12	35.5	0.176	192	115	1071	4.8	6.6	0.17
54	0.064	3.19	24.885	0.142	185	143.5	1018	5.15	6.65	0.17
55	0.176	3.63	41.259	0.1595	158.5	111	844.5	5.4	6.55	0.17
57	0.081	5.28	36.396	0.224	151	93	718	5.1	6.6	0.16

Table A5. Continued

Plot	Na (ppm)	CEC†	% Sand	% Silt	% Clay	Texture
30	8	9.7	59.2	28	12.8	Sandy Loam
31	7	8.9	57.2	30	12.8	Sandy Loam
32	8	18.2	69.2	24	6.8	Sandy Loam
33	7	11.3	63.2	24	12.8	Sandy Loam
34	6	15.1	63.2	28	8.8	Sandy Loam
35	4	15.2	69.2	20	10.8	Sandy Loam
36	6	13.1	65.2	28	6.8	Sandy Loam
37	8	17.9	55.2	30	14.8	Sandy Loam
38	10	12.3	65.2	26	8.8	Sandy Loam
39	5	13.9	63.2	24	12.8	Sandy Loam
40	7	13.6	71.2	22	6.8	Sandy Loam
42	6	10.4	61.2	26	12.8	Sandy Loam
43	8	15.7	69.2	22	8.8	Sandy Loam
44	6	13.2	67.2	22	10.8	Sandy Loam
45	7	11.7	63.2	26	10.8	Sandy Loam
46	2	11.9	65.2	22	12.8	Sandy Loam
47	3	11.2	69.2	24	6.8	Sandy Loam
48	3	12	67.2	22	10.8	Sandy Loam
50	6	14.5	63.2	28	8.8	Sandy Loam
51	2	17.2	61.2	20	18.8	Sandy Loam
52	6	13	45.2	40	14.8	Loam
53	7	11.8	63.2	18	18.8	Sandy Loam
54	8.5	11.3	70.2	21.2	8.6	Sandy Loam
55	7	11.1	70.2	13	16.8	Sandy Loam
57	8	9.8	61.2	30	8.8	Sandy Loam

Table A5. Continued

Plot	Soil $\delta^{15}N$ (‰)	Soil % N	% Soil Moisture	Potential NO_3 (ppm)	Potential NH_4 (ppm)	Total Potential Nitrification (ppm)	Mineral NO_3 (ppm)	Mineral NH_4 (ppm)
58	-3.48	0.174	17.81	0.103	0.096	0.199	-0.010	0.134
59	-2.44	0.174	10.51	0.140	0.213	0.353	-0.070	-0.007
60	-0.86	0.322	19.74	0.015	0.166	0.180	0.022	0.360
61	-2.86	0.135	18.04	-0.041	-1.065	-1.106	0.085	1.278
62	-1.01	0.221	25.60	0.238	0.737	0.974	-0.043	0.647
63	-6.47	0.096	23.29	0.019	-0.039	-0.019	0.049	0.282
64	-2.41	0.119	14.83	0.002	0.031	0.033	0.052	0.094
65	-3.54	0.099	11.30	0.037	0.077	0.114	0.038	0.058
66	-2.64	0.171	16.90	0.025	0.015	0.040	0.016	0.192

Plot	Total Mineral N (ppm)	Total % C	Bray P (ppm)	% TKN	K (ppm)	Mg (ppm)	Ca (ppm)	pH	Buffer pH	Soluble Salts
58	0.124	3.65	44.731	0.1455	212	128	1094	5.7	6.85	0.15
59	-0.076	4.93	38.023	0.168	182	140	1126	5.2	6.5	0.19
60	0.382	6.38	81.744	0.278	209	107	1332	5.5	6.8	0.16
61	1.363	3.60	66.245	0.139	145	87	714	5.6	6.8	0.13
62	0.604	5.63	38.546	0.251	169	150	1078	5.4	6.4	0.2
63	0.331	3.48	56.533	0.146	191	153	972	5.1	6.4	0.19
64	0.146	4.35	58.872	0.158	141	77	552	5.2	6.7	0.13
65	0.096	1.97	41.471	0.106	108	71	490	4.7	6.5	0.15
66	0.208	3.89	23.397	0.141	109	75	675	4.6	6.2	0.19

Table A5. Continued

Plot	Na (ppm)	CEC†	% Sand	% Silt	% Clay	Texture
58	5.5	9.7	61.2	28	10.8	Sandy Loam
59	8	13.3	63.2	30	6.8	Sandy Loam
60	7	10.9	71.2	22	6.8	Sandy Loam
61	6	7.5	61.2	28	10.8	Sandy Loam
62	8	14.1	63.2	30	6.8	Sandy Loam
63	9	13.7	57.2	30	12.8	Sandy Loam
64	7	7.8	63.2	32	4.8	Sandy Loam
65	6	9.3	71.2	22	6.8	Sandy Loam
66	7	13.3	59.2	34	6.8	Sandy Loam

*TKN = Total N determined using the kjedahl digestion method
†CEC= Cation exchange capacity

Table A6. Pearson correlation coefficients, *P*-values, and number of observations for environmental, vegetation, $\delta^{15}N$, and soil data. The best 15 correlations are shown for each variable. The first row is the correlation coefficient, the second row is Prob > |r| under H_0: Rho=0, and the last row is *n*. Using Dinn-Sidak correction $\alpha' = 0.0034$.

	Best 1	**Best 2**	**Best 3**	**Best 4**
Location Code[a]	Location Code	Na (ppm)	Alive lodgepole (BA)	Alive:Dead[b]
	1	-0.62471	-0.59918	-0.48593
		<.0001	<.0001	0.0003
	59	59	59	50
Elevation	Elevation	Location Code	Relative PAR[c]	Dead lodgepole (BA)
	1	0.40893	0.3671	0.34361
		0.0013	0.0042	0.0077
	59	59	59	59
Aspect	Aspect	Elevation	Potential NO_3[d]	Total potential nitrification[e]
	1	-0.26314	0.19939	0.19265
		0.044	0.13	0.1438
	59	59	59	59
Slope	Slope	% Sand	Elevation	Alive lodgepole (BA)
	1	0.28036	0.26839	-0.24727
		0.0315	0.0398	0.059
	59	59	59	59
Berries (m^2)	Berries (m^2)	Buffaloberry cover	Dead lodgepole (BA)	Alive:Dead
	1	0.59757	0.26453	-0.24217
		<.0001	0.0429	0.0902
	59	59	59	50
N_2 Fixer Present[f]	N_2 Fixer Present	Na (ppm)	% Soil moisture	Ca (ppm)
	1	0.46553	0.39372	0.34312
		0.0002	0.002	0.0078
	59	59	59	59
Alive lodgepole (BA[g]**)**	Alive lodgepole (BA)	Alive:Dead	Location Code	Total basal area
	1	0.82901	-0.59918	0.5749
		<.0001	<.0001	<.0001
	59	50	59	59

Table A6. Continued

	Best 5	Best 6	Best 7	Best 8
Location Code	Elevation	Total basal area	Relative PAR	% Sand
	0.40893	-0.39522	0.38455	0.35575
	0.0013	0.0019	0.0026	0.0057
	59	59	59	59
Elevation	δ^{15}N rose[h]	Bray P (ppm)	Alive lodgepole (BA)	Slope
	-0.30797	-0.30582	-0.2859	0.26839
	0.0176	0.0185	0.0282	0.0398
	59	59	59	59
Aspect	% Silt	Na (ppm)	Mg (ppm)	Berries (m^2)
	-0.18975	-0.16713	0.16148	0.15131
	0.15	0.2058	0.2218	0.2526
	59	59	59	59
Slope	N_2 Fixer Present	δ^{15}N rose	Na (ppm)	Reference species %N
	-0.24018	0.23839	-0.23153	-0.22908
	0.0669	0.069	0.0777	0.0809
	59	59	59	59
Berries (m^2)	Mineral NH_4[i]	Total mineral N[j]	Na (ppm)	Location Code
	0.24195	0.23634	-0.21567	0.2125
	0.0649	0.0715	0.1009	0.1061
	59	59	59	59
N_2 Fixer Present	Mineral NH_4	% TKN[k]	% Sand	Location Code
	0.31846	0.31411	-0.3038	-0.27676
	0.014	0.0154	0.0193	0.0338
	59	59	59	59
Alive lodgepole (BA)	Relative PAR	Na (ppm)	Mineral NH_4	Dead lodgepole (BA)
	-0.42048	0.36783	-0.33523	-0.33105
	0.0009	0.0042	0.0094	0.0104
	59	59	59	59

Table A6. Continued

	Best 9	Best 10	Best 11	Best 12
Location Code	% Silt	Buffaloberry foliar %N	Buffer pH	Reference species %N
	-0.33692	-0.33334	-0.31866	0.27875
	0.0091	0.0099	0.0139	0.0325
	59	59	59	59
Elevation	δ^{15}N Soil	Aspect	% Silt	N^{2} Fixer Present
	-0.26811	-0.26314	-0.2613	-0.24265
	0.0419	0.044	0.0456	0.0641
	58	59	59	59
Aspect	% Clay	K (ppm)	Ca (ppm)	Potential NH_4^1
	0.1374	0.13715	0.13435	0.13208
	0.2994	0.3003	0.3104	0.3187
	59	59	59	59
Slope	% Clay	% Silt	Total basal area	Location Code
	-0.20106	-0.19949	-0.195	0.19185
	0.1268	0.1298	0.1389	0.1455
	59	59	59	59
Berries (m^2)	Alive lodgepole (BA)	N_2 Fixer Present	Aspect	Foliar δ^{15}N buffaloberry
	-0.19961	-0.16258	0.15131	-0.12692
	0.1296	0.2186	0.2526	0.3381
	59	59	59	59
N_2 Fixer Present	Dead lodgepole (BA)	Mg (ppm)	Total mineral N	N_{dfa}^m
	-0.27379	0.27105	0.26845	0.26717
	0.0359	0.0379	0.0398	0.0408
	59	59	59	59
Alive lodgepole (BA)	Buffaloberry cover	Total mineral N	N_{dfa}	Elevation
	-0.31045	-0.29685	-0.29419	-0.2859
	0.0167	0.0224	0.0237	0.0282
	59	59	59	59

Table A6. Continued

	Best 13	Best 14	Best 15
Location Code	N_2 Fixer Present	Mineral NH_4	% Soil moisture
	-0.27676	0.26453	-0.26152
	0.0338	0.0429	0.0454
	59	59	59
Elevation	Reference species %N	Na (ppm)	% Sand
	0.23929	-0.23355	0.20702
	0.068	0.075	0.1157
	59	59	59
Aspect	$\delta^{15}N$ all reference species[n]	% Sand	Location Code
	-0.12797	0.10427	0.09832
	0.3341	0.4319	0.4588
	59	59	59
Slope	Alive:Dead	% Soil moisture	Buffer pH
	-0.18455	-0.17696	0.14604
	0.1995	0.18	0.2697
	50	59	59
Berries (m^2)	Potential NO_3	% Soil moisture	Total C
	-0.12413	0.11322	0.10447
	0.3489	0.3932	0.431
	59	59	59
N_2 Fixer Present	% Silt	Elevation	Slope
	0.25402	-0.24265	-0.24018
	0.0522	0.0641	0.0669
	59	59	59
Alive lodgepole (BA)	Reference species %N	CEC[o]	Ca (ppm)
	-0.27519	-0.26845	-0.265
	0.0349	0.0398	0.0425
	59	59	59

Table A6. Continued

	Best 1	Best 2	Best 3	Best 4
Dead lodgepole (BA)	Dead lodgepole (BA)	Buffaloberry cover	Total basal area	Alive:Dead
	1	0.54808	0.47483	-0.41339
		<.0001	0.0001	0.0028
	59	59	59	50
Alive:Dead	Alive:Dead	Alive lodgepole (BA)	Location Code	Total basal area
	1	0.82901	-0.48593	0.43755
		<.0001	0.0003	0.0015
	50	50	50	50
Total basal area	Total basal area	Alive lodgepole (BA)	Relative PAR	Dead lodgepole (BA)
	1	0.5749	-0.51789	0.47483
		<.0001	<.0001	0.0001
	59	59	59	59
Relative PAR	Relative PAR	Total basal area	Alive lodgepole (BA)	% Soil moisture
	1	-0.51789	-0.42048	-0.3967
		<.0001	0.0009	0.0019
	59	59	59	59
Buffaloberry cover	Buffaloberry cover	Berries (m^2)	Dead lodgepole (BA)	Alive:Dead
	1	0.59757	0.54808	-0.39859
		<.0001	<.0001	0.0041
	59	59	59	50
δ^{15}N rose	δ^{15}N rose	δ^{15}N all reference species	Mineral NO_3	Relative PAR
	1	0.78269	0.3573	-0.31766
		<.0001	0.0055	0.0142
	59	59	59	59
Foliar δ15N buffaloberry	Foliar δ15N buffaloberry	Buffaloberry foliar %N	N_{dfa}	% Soil moisture
	1	0.43904	0.30569	0.28159
		0.0005	0.0185	0.0307
	59	59	59	59

Table A6. Continued.

	Best 1	**Best 2**	**Best 3**	**Best 4**
δ^{15}N all reference species	δ^{15}N all reference species	δ^{15}N rose	Total basal area	Relative PAR
	1	0.78269	0.32666	-0.32293
		<.0001	0.0116	0.0126
	59	59	59	59
N_{dfa}	N_{dfa}	CEC	Ca (ppm)	Mg (ppm)
	1	0.49772	0.49289	0.49079
		<.0001	<.0001	<.0001
	59	59	59	59
Buffaloberry foliar %N	Buffaloberry foliar %N	% Soil moisture	Na (ppm)	N_{dfa}
	1	0.64153	0.48101	0.47859
		<.0001	0.0001	0.0001
	59	59	59	59
Reference species %N	Reference species %N	Buffer pH	CEC	Location Code
	1	-0.36036	0.31858	0.27875
		0.0051	0.0139	0.0325
	59	59	59	59
δ^{15}N Soil	δ^{15}N Soil	Ca (ppm)	K (ppm)	Mg (ppm)
	1	0.47293	0.44425	0.4336
		0.0002	0.0005	0.0007
	58	58	58	58
Soil %N	Soil %N	Total C	% TKN	CEC
	1	0.90375	0.90298	0.60424
		<.0001	<.0001	<.0001
	58	58	58	58
% Soil moisture	% Soil moisture	Buffaloberry foliar %N	% Sand	Total C
	1	0.64153	-0.61392	0.60658
		<.0001	<.0001	<.0001
	59	59	59	59

Table A6. Continued

	Best 5	**Best 6**	**Best 7**	**Best 8**
δ^{15}N all reference species	Mineral NO_3	Potential NO_3	Total potential nitrification	Dead lodgepole (BA)
	0.29823	-0.2674	-0.24725	0.20253
	0.0218	0.0406	0.059	0.124
	59	59	59	59
N_{dfa}	Buffaloberry foliar %N	Soil %N	% Clay	% TKN
	0.47859	0.43739	0.43545	0.35256
	0.0001	0.0006	0.0006	0.0062
	59	58	59	59
Buffaloberry foliar %N	Foliar δ15N buffaloberry	% Sand	Mg (ppm)	Ca (ppm)
	0.43904	-0.41863	0.40904	0.40505
	0.0005	0.001	0.0013	0.0015
	59	59	59	59
Reference species %N	Alive lodgepole (BA)	Mg (ppm)	Elevation	Buffaloberry foliar %N
	-0.27519	0.2487	0.23929	0.23154
	0.0349	0.0575	0.068	0.0776
	59	59	59	59
δ^{15}N Soil	% TKN	CEC	N_{dfa}	Soil %N
	0.35429	0.31748	0.30371	0.30039
	0.0064	0.0152	0.0205	0.022
	58	58	58	58
Soil %N	Ca (ppm)	% Soil moisture	N_{dfa}	Mg (ppm)
	0.58262	0.54382	0.43739	0.43688
	<.0001	<.0001	0.0006	0.0006
	58	58	58	58
% Soil moisture	% TKN	Na (ppm)	Soil %N	% Silt
	0.59998	0.54811	0.54382	0.49326
	<.0001	<.0001	<.0001	<.0001
	59	59	58	59

Table A6. Continued

	Best 9	Best 10	Best 11	Best 12
δ^{15}N all reference species	Buffaloberry cover	Total mineral N	Potential NH_4	% Soil moisture
	0.19136	0.18882	-0.16404	0.15741
	0.1465	0.1521	0.2144	0.2338
	59	59	59	59
N_{dfa}	% Soil moisture	Foliar δ15N buffaloberry	δ^{15}N Soil	Alive lodgepole (BA)
	0.33572	0.30569	0.30371	-0.29419
	0.0093	0.0185	0.0205	0.0237
	59	59	58	59
Buffaloberry foliar %N	CEC	Relative PAR	% Clay	% TKN
	0.402	-0.38555	0.36009	0.35163
	0.0016	0.0026	0.0051	0.0063
	59	59	59	59
Reference species %N	Slope	Foliar δ15N buffaloberry	Ca (ppm)	% Soil moisture
	-0.22908	0.19196	0.18663	0.17571
	0.0809	0.1452	0.157	0.1831
	59	59	59	59
δ^{15}N Soil	Elevation	Total C	Na (ppm)	% Sand
	-0.26811	0.22612	0.22059	-0.21363
	0.0419	0.0879	0.0961	0.1074
	58	58	58	58
Soil %N	K (ppm)	Buffer pH	% Clay	δ^{15}N Soil
	0.36342	-0.32908	0.32093	0.30039
	0.005	0.0117	0.014	0.022
	58	58	58	58
% Soil moisture	CEC	Relative PAR	N_2 Fixer Present	Ca (ppm)
	0.43198	-0.3967	0.39372	0.37206
	0.0006	0.0019	0.002	0.0037
	59	59	59	59

Table A6. Continued

	Best 13	Best 14	Best 15
δ^{15}N all reference species	Reference species %N	δ^{15}N Soil	% TKN
	0.15708	-0.14173	-0.13819
	0.2348	0.2886	0.2966
	59	58	59
N_{dfa}	Total C	N_2 Fixer Present	Buffer pH
	0.28868	0.26717	-0.22311
	0.0266	0.0408	0.0894
	59	59	59
Buffaloberry foliar %N	Location Code	Alive:Dead	Soil %N
	-0.33334	0.29713	0.29248
	0.0099	0.0361	0.0259
	59	50	58
Reference species %N	% Clay	δ^{15}N all reference species	Dead lodgepole (BA)
	0.1624	0.15708	0.15649
	0.2191	0.2348	0.2366
	59	59	59
δ^{15}N Soil	% Clay	Dead lodgepole (BA)	Foliar δ15N buffaloberry
	0.20341	-0.1898	0.17627
	0.1257	0.1536	0.1856
	58	58	58
Soil %N	Buffaloberry foliar %N	% Sand	Na (ppm)
	0.29248	-0.25587	0.25101
	0.0259	0.0525	0.0574
	58	58	58
% Soil moisture	% Clay	N_{dfa}	Mg (ppm)
	0.33667	0.33572	0.32513
	0.0091	0.0093	0.012
	59	59	59

Table A6. Continued

	Best 1	Best 2	Best 3	Best 4
Potential NO_3	Potential NO_3	Total potential nitrification	Relative PAR	Total basal area
	1	0.54765	0.3684	-0.32491
		<.0001	0.0041	0.012
	59	59	59	59
Potential NH_4	Potential NH_4	Total potential nitrification	Total mineral N	Mineral NH_4
	1	0.91739	-0.47991	-0.44166
		<.0001	0.0001	0.0005
	59	59	59	59
Total potential nitrification	Total potential nitrification	Potential NH_4	Potential NO_3	Total mineral N
	1	0.91739	0.54765	-0.44759
		<.0001	<.0001	0.0004
	59	59	59	59
Mineral NO_3	Mineral NO_3	Total mineral N	Total potential nitrification	Mineral NH_4
	1	0.58338	-0.44185	0.40668
		<.0001	0.0005	0.0014
	59	59	59	59
Mineral NH_4	Mineral NH_4	Total mineral N	Potential NH_4	K (ppm)
	1	0.97925	-0.44166	0.41319
		<.0001	0.0005	0.0011
	59	59	59	59
Total mineral N	Total mineral N	Mineral NH_4	Mineral NO_3	Potential NH_4
	1	0.97925	0.58338	-0.47991
		<.0001	<.0001	0.0001
	59	59	59	59
Total C	Total C	% TKN	Soil %N	% Soil moisture
	1	0.9345	0.90375	0.60658
		<.0001	<.0001	<.0001
	59	59	58	59

Table A6. Continued

	Best 5	Best 6	Best 7	Best 8
Potential NO_3	$\delta^{15}N$ rose	$\delta^{15}N$ all reference species	Mineral NO_3	Alive:Dead
	-0.29434	-0.2674	-0.26726	-0.26237
	0.0236	0.0406	0.0407	0.0657
	59	59	59	50
Potential NH_4	Mineral NO_3	Total basal area	Mg (ppm)	CEC
	-0.39333	0.27847	0.21707	0.2158
	0.0021	0.0327	0.0987	0.1007
	59	59	59	59
Total potential nitrification	Mineral NO_3	Mineral NH_4	$\delta^{15}N$ rose	Bray P (ppm)
	-0.44185	-0.3932	-0.28994	-0.25638
	0.0005	0.0021	0.0259	0.05
	59	59	59	59
Mineral NO_3	Potential NH_4	$\delta^{15}N$ rose	% Sand	% Silt
	-0.39333	0.3573	-0.31266	0.30225
	0.0021	0.0055	0.0159	0.02
	59	59	59	59
Mineral NH_4	Mineral NO_3	Total potential nitrification	Bray P (ppm)	Total basal area
	0.40668	-0.3932	0.3871	-0.35893
	0.0014	0.0021	0.0025	0.0052
	59	59	59	59
Total mineral N	Total potential nitrification	K (ppm)	Bray P (ppm)	Total basal area
	-0.44759	0.40009	0.39592	-0.30835
	0.0004	0.0017	0.0019	0.0175
	59	59	59	59
Total C	CEC	Ca (ppm)	Buffer pH	% Sand
	0.48462	0.39183	-0.38567	-0.31275
	0.0001	0.0021	0.0026	0.0159
	59	59	59	59

Table A6. Continued

	Best 9	Best 10	Best 11	Best 12
Potential NO_3	% Soil moisture	% Sand	Na (ppm)	Bray P (ppm)
	-0.24704	0.23881	-0.23119	-0.22707
	0.0593	0.0685	0.0781	0.0837
	59	59	59	59
Potential NH_4	$\delta^{15}N$ rose	Bray P (ppm)	Potential NO_3	$\delta^{15}N$ all reference species
	-0.20152	-0.19399	0.16942	-0.16404
	0.1259	0.141	0.1996	0.2144
	59	59	59	59
Total potential nitrification	$\delta^{15}N$ all reference species	Aspect	% Silt	Mg (ppm)
	-0.24725	0.19265	-0.18194	0.17399
	0.059	0.1438	0.1679	0.1875
	59	59	59	59
Mineral NO_3	$\delta^{15}N$ all reference species	Potential NO_3	% Soil moisture	Alive:Dead
	0.29823	-0.26726	0.24785	0.23757
	0.0218	0.0407	0.0584	0.0967
	59	59	59	50
Mineral NH_4	Alive lodgepole (BA)	N_2 Fixer Present	Buffaloberry cover	% Soil moisture
	-0.33523	0.31846	0.29361	0.2658
	0.0094	0.014	0.024	0.0419
	59	59	59	59
Total mineral N	Buffaloberry cover	Alive lodgepole (BA)	% Soil moisture	% Sand
	0.29822	-0.29685	0.29128	-0.27034
	0.0218	0.0224	0.0252	0.0384
	59	59	59	59
Total C	Ndfa	Mg (ppm)	Buffaloberry foliar %N	Relative PAR
	0.28868	0.27883	0.27523	-0.26936
	0.0266	0.0325	0.0349	0.0391
	59	59	59	59

Table A6. Continued

	Best 13	Best 14	Best 15
Potential NO_3	% Silt	Aspect	Alive lodgepole (BA)
	-0.21628	0.19939	-0.18303
	0.0999	0.13	0.1653
	59	59	59
Potential NH_4	Buffer pH	N_2 Fixer Present	Ca (ppm)
	-0.16233	-0.16206	0.14862
	0.2193	0.2201	0.2613
	59	59	59
Total potential nitrification	% Sand	CEC	Elevation
	0.17159	0.15258	0.14481
	0.1938	0.2486	0.2738
	59	59	59
Mineral NO_3	Bray P (ppm)	Buffaloberry foliar %N	Buffaloberry cover
	0.23339	0.17564	0.16766
	0.0752	0.1833	0.2044
	59	59	59
Mineral NH_4	Location Code	Ca (ppm)	Alive:Dead
	0.26453	0.24427	-0.24393
	0.0429	0.0623	0.0878
	59	59	50
Total mineral N	N_2 Fixer Present	Location Code	Berries (m^2)
	0.26845	0.25268	0.23634
	0.0398	0.0535	0.0715
	59	59	59
Total C	% Clay	Na (ppm)	K (ppm)
	0.26516	0.2598	0.23938
	0.0424	0.0469	0.0678
	59	59	59

Table A6. Continued

	Best 1	Best 2	Best 3	Best 4
Bray P (ppm)	Bray P (ppm)	Total mineral N	Mineral NH_4	Elevation
	1	0.39592	0.3871	-0.30582
		0.0019	0.0025	0.0185
	59	59	59	59
% TKN	% TKN	Total C	Soil %N	% Soil moisture
	1	0.9345	0.90298	0.59998
		<.0001	<.0001	<.0001
	59	59	58	59
K (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	CEC
	1	0.74008	0.73063	0.70792
		<.0001	<.0001	<.0001
	59	59	59	59
Mg (ppm)	Mg (ppm)	Ca (ppm)	CEC	K (ppm)
	1	0.9275	0.86687	0.73063
		<.0001	<.0001	<.0001
	59	59	59	59
Ca (ppm)	Ca (ppm)	Mg (ppm)	CEC	K (ppm)
	1	0.9275	0.86401	0.74008
		<.0001	<.0001	<.0001
	59	59	59	59
Buffer pH	Buffer pH	CEC	Total C	Reference species %N
	1	-0.64228	-0.38567	-0.36036
		<.0001	0.0026	0.0051
	59	59	59	59
Na (ppm)	Na (ppm)	Location Code	% Soil moisture	% Sand
	1	-0.62471	0.54811	-0.51402
		<.0001	<.0001	<.0001
	59	59	59	59

Table A6. Continued

	Best 5	Best 6	Best 7	Best 8
Bray P (ppm)	Total potential nitrification	K (ppm)	Mineral NO_3	Potential NO_3
	-0.25638	0.25128	0.23339	-0.22707
	0.05	0.0549	0.0752	0.0837
	59	59	59	59
% TKN	CEC	Ca (ppm)	$\delta^{15}N$ Soil	N_{dfa}
	0.49953	0.49807	0.35429	0.35256
	<.0001	<.0001	0.0064	0.0062
	59	59	58	59
K (ppm)	% Clay	$\delta^{15}N$ Soil	Mineral NH_4	Total mineral N
	0.51719	0.44425	0.41319	0.40009
	<.0001	0.0005	0.0011	0.0017
	59	58	59	59
Mg (ppm)	% Clay	N_{dfa}	Soil %N	δ15N Soil
	0.62885	0.49079	0.43688	0.4336
	<.0001	<.0001	0.0006	0.0007
	59	59	58	58
Ca (ppm)	% Clay	Soil %N	% TKN	N_{dfa}
	0.62516	0.58262	0.49807	0.49289
	<.0001	<.0001	<.0001	<.0001
	59	58	59	59
Buffer pH	Soil %N	Location Code	% Soil moisture	Mg (ppm)
	-0.32908	-0.31866	-0.30272	-0.26833
	0.0117	0.0139	0.0198	0.0399
	58	59	59	59
Na (ppm)	Buffaloberry foliar %N	N_2 Fixer Present	Alive:Dead	% Silt
	0.48101	0.46553	0.4316	0.40655
	0.0001	0.0002	0.0018	0.0014
	59	59	50	59

Table A6. Continued

	Best 9	Best 10	Best 11	Best 12
Bray P (ppm)	Potential NH_4	$\delta^{15}N$ rose	Alive:Dead	Buffaloberry cover
	-0.19399	0.19359	-0.19084	0.14084
	0.141	0.1418	0.1843	0.2873
	59	59	50	59
% TKN	Buffaloberry foliar %N	Mg (ppm)	K (ppm)	N_2 Fixer Present
	0.35163	0.3431	0.31776	0.31411
	0.0063	0.0078	0.0142	0.0154
	59	59	59	59
K (ppm)	Soil %N	% TKN	Buffaloberry foliar %N	Buffer pH
	0.36342	0.31776	0.27208	-0.26611
	0.005	0.0142	0.0371	0.0416
	58	59	59	59
Mg (ppm)	Buffaloberry foliar %N	Na (ppm)	% TKN	% Soil moisture
	0.40904	0.38916	0.3431	0.32513
	0.0013	0.0023	0.0078	0.012
	59	59	59	59
Ca (ppm)	$\delta^{15}N$ Soil	Buffaloberry foliar %N	Total C	% Soil moisture
	0.47293	0.40505	0.39183	0.37206
	0.0002	0.0015	0.0021	0.0037
	58	59	59	59
Buffer pH	K (ppm)	% TKN	$\delta^{15}N$ rose	N_{dfa}
	-0.26611	-0.25026	0.24951	-0.22311
	0.0416	0.0559	0.0567	0.0894
	59	59	59	59
Na (ppm)	Mg (ppm)	Alive lodgepole (BA)	Ca (ppm)	Relative PAR
	0.38916	0.36783	0.35377	-0.3259
	0.0023	0.0042	0.006	0.0118
	59	59	59	59

Table A6. Continued

	Best 13	Best 14	Best 15
Bray P (ppm)	N_{dfa}	Relative PAR	$\delta^{15}N$ all reference species
	-0.14036	-0.13845	0.12577
	0.289	0.2957	0.3425
	59	59	59
% TKN	% Sand	Na (ppm)	% Clay
	-0.30705	0.30555	0.29451
	0.018	0.0186	0.0236
	59	59	59
K (ppm)	Bray P (ppm)	% Soil moisture	Total C
	0.25128	0.24304	0.23938
	0.0549	0.0636	0.0678
	59	59	59
Mg (ppm)	Total C	N_2 Fixer Present	Buffer pH
	0.27883	0.27105	-0.26833
	0.0325	0.0379	0.0399
	59	59	59
Ca (ppm)	Na (ppm)	N_2 Fixer Present	Alive lodgepole (BA)
	0.35377	0.34312	-0.265
	0.006	0.0078	0.0425
	59	59	59
Buffer pH	Foliar δ15N buffaloberry	% Clay	Dead lodgepole (BA)
	-0.21065	-0.18944	-0.18803
	0.1093	0.1507	0.1538
	59	59	59
Na (ppm)	Total basal area	% TKN	% Clay
	0.31627	0.30555	0.29371
	0.0147	0.0186	0.024
	59	59	59

Table A6. Continued

	Best 1	Best 2	Best 3	Best 4
CEC	CEC	Mg (ppm)	Ca (ppm)	K (ppm)
	1	0.86687	0.86401	0.70792
		<.0001	<.0001	<.0001
	59	59	59	59
% Sand	% Sand	% Silt	% Soil moisture	Na (ppm)
	1	-0.87016	-0.61392	-0.51402
		<.0001	<.0001	<.0001
	59	59	59	59
% Silt	% Silt	% Sand	% Soil moisture	Na (ppm)
	1	-0.87016	0.49326	0.40655
		<.0001	<.0001	0.0014
	59	59	59	59
% Clay	% Clay	Mg (ppm)	Ca (ppm)	CEC
	1	0.62885	0.62516	0.5791
		<.0001	<.0001	<.0001
	59	59	59	59

Table A6. Continued

	Best 5	Best 6	Best 7	Best 8
CEC	Buffer pH	Soil %N	% Clay	% TKN
	-0.64228	0.60424	0.5791	0.49953
	<.0001	<.0001	<.0001	<.0001
	59	58	59	59
% Sand	% Clay	Buffaloberry foliar %N	Location Code	Total C
	-0.42593	-0.41863	0.35575	-0.31275
	0.0008	0.001	0.0057	0.0159
	59	59	59	59
% Silt	Location Code	Mineral NO_3	Relative PAR	Buffaloberry foliar %N
	-0.33692	0.30225	-0.26986	0.26527
	0.0091	0.02	0.0387	0.0423
	59	59	59	59
% Clay	K (ppm)	N_{dfa}	% Sand	Buffaloberry foliar %N
	0.51719	0.43545	-0.42593	0.36009
	<.0001	0.0006	0.0008	0.0051
	59	59	59	59

Table A6. Continued

	Best 9	Best 10	Best 11	Best 12
CEC	N_{dfa}	Total C	% Soil moisture	Buffaloberry foliar %N
	0.49772	0.48462	0.43198	0.402
	<.0001	0.0001	0.0006	0.0016
	59	59	59	59
% Sand	Mineral NO_3	% TKN	N_2 Fixer Present	Slope
	-0.31266	-0.30705	-0.3038	0.28036
	0.0159	0.018	0.0193	0.0315
	59	59	59	59
% Silt	Elevation	N_2 Fixer Present	Alive:Dead	Potential NO_3
	-0.2613	0.25402	0.23471	-0.21628
	0.0456	0.0522	0.1009	0.0999
	59	59	50	59
% Clay	% Soil moisture	Soil %N	% TKN	Na (ppm)
	0.33667	0.32093	0.29451	0.29371
	0.0091	0.014	0.0236	0.024
	59	58	59	59

Table A6. Continued

	Best 13	Best 14	Best 15
CEC	Reference species %N	δ^{15}N Soil	Na (ppm)
	0.31858	0.31748	0.28184
	0.0139	0.0152	0.0306
	59	58	59
% Sand	Alive:Dead	Total mineral N	Ca (ppm)
	-0.27517	-0.27034	-0.26108
	0.0531	0.0384	0.0458
	50	59	59
% Silt	Total C	Slope	Aspect
	0.20027	-0.19949	-0.18975
	0.1283	0.1298	0.15
	59	59	59
% Clay	Total C	Mineral NH_4	Total mineral N
	0.26516	0.2124	0.20608
	0.0424	0.1063	0.1174
	59	59	59

[a] Location code = Plots were separated by area into 9 categorical codes.
[b] Alive:Dead = The ratio of alive to dead lodge pole pine as measured by basal area
[c] PAR = Photosynthetically active radiation
[d] Potenital NO_3 = Potential soil nitrification (ppm)
[e] Total potential nitrification = Total potential soil nitrification (ppm)
[f] N_2 fixer present = Presence/absence of legume species (Nevada pea (*Lathyrus lanszwertii* Kellogg var. *leucanthus* (Rydb.) Dorn), silvery lupine (*Lupinus argenteus* Pursh), and goldenbanner (*Thermopsis divaricarpa* A. Nelson)) and other actinorhizal shrubs (Snowbrush (*Ceanothus velutinus* Douglas ex Hook))
[g] BA = Basal area estimated using a 20 basal area factor glass prism
[h] δ^{15}N rose = Foliar δ^{15}N of the reference species Wood's rose (*Rosa woodsii*)
[i] Mineral NH_4 = Mineral soil NH_4 (ppm)
[j] Total mineral N = Total soil mineral N (ppm)
[k] TKN = Total % N determined using the kjedahl digestion method
[l] Potential NH_4 = Potential soil NH_4 (ppm)
[m] N_{dfa} = N derived from N_2-fixation
[n] δ^{15}N all reference species = average δ^{15}N of all reference species across all plots
[o] CEC= Cation exchange capacity
Species: Buffaloberry (*Shepherdia canadensis*)

APPENDIX 2

The trials and tribulations of a greenhouse study with buffaloberry

The goal of the greenhouse study was to compliment the field data with a controlled experiment to determine if shading and plant available N affect N_2-fixation in buffaloberry. For the experiment I needed 300 shrubs to have adequate replications. Propagation from live cuttings provides many replicates at a low cost. On November 8, 2008 I, along with 3 members of the Restoration Ecology Laboratory (REL), collected over 1,000 cuttings from 30 individual buffaloberry plants. 2.5 kg of soil was also sampled underneath several buffaloberry shrubs to be used for inoculum of *Frankia*. Each 10-13 cm long stem was cut at a 45° angel at the end of buffaloberry stems. The cuttings were stored standing up, covered in snow to keep them cool, in a large cooler ¼ full of wet shredded paper. Immediately upon returning to Colorado State University (CSU), the bottom 0.6 cm of the cut end of each cutting was dipped Root-tone© and planted in a cone-tainer filled with general potting soil. The racks of cone-tainers were placed on the misting bench on heating pads in the conservatory of the Plant Growth Facility greenhouse at CSU. None of these cuttings survived.

On March 5, 2009 an additional 1,000 buffaloberry cuttings were collected and transported as above. The cut end was dipped in Rhizopon AA powder rooting hormone and planted in flats using an N-free planting media. A soil and N free planting media was mixed from 3 parts sand, 2 parts vermiculite, and 1 part perlite. I used this media mixture because the study needed an N-free medium so ^{15}N could be monitored more easily, but also because the first batch of cuttings rotted and I assumed there was not enough drainage in the soil to promote root formation. The flats were placed on heating pads under an automated misting system that misted for 30 seconds every 5 minutes during daylight hours. After 3 weeks, the misting system was adjusted to mist every 10 minutes as the conditions were too moist. Two weeks after planting, the flats were treated with an inoculum slurry made from mixing water with the native soils collected under buffaloberry shrubs. In one month only 120 cuttings had formed roots so on

April 21, 2009 the cuttings were treated with a foliar application of a synthetic auxin IBA rooting hormone diluted to 1,000 ppm. As cuttings were found with roots, they were transferred into large cone-tainers using the same planting media and inoculated using 15-30 grams of the native soil slurry for each plant. They were watered after inoculation. By May 2009, there were only 200 alive rooted cuttings out of 1,000.

To propagate buffaloberry from cuttings, I would advise using early spring cuttings that have buds. With buds already formed, the cuttings would have more energy to put into rooting and nodule formation. I would also advise using a sandy soil media that does not retain a lot of moisture and watering regime that allows for the soil to dry out between watering. Finally, I believe that any temperature in the greenhouse above 24 degrees C may not be optimal conditions for russet buffaloberry. Ideally, if I could run this study again I would find a protected outdoor area to conduct the study.

In July 2009, 360 buffaloberry shrubs were purchased from Rocky Mountain Native Plants Company (RMNP) in Rifle, CO. These shrubs were in 3.8 L pots and most had well-formed nodules. However, they had been growing in these pots for at least 2 years in an outdoor greenhouse at RMNP. I transferred them into clean 3.8 L pots with the N-free potting media and inoculated them again using the native soil slurry. Almost immediately upon arrival 20 plants perished. On July 22, 2009 I began fertilizing the plants with an N-free nutrient solution (Table A7) to encourage nodulation and to avoid adding unlabelled N to the plants before initiating the study. The fertilizer schedule involved watering with the N-free fertilizer one day, the Ca only fertilizer the next day, followed by no water on the third day. I also inoculated the plants 2 more times with the native soil slurry weekly to ensure *Frankia* availability. By the end of July almost all the shrubs showed signs of nutrient deficiency and shock from moving inside to a hot greenhouse. On August 6, 2009 I incorporated an N fertilizer into the schedule as the plants' health continued to decline. By the end of August the plants were in poor condition. With the intensive fertilizing regime, I thought that the pots were not given enough time to dry out between

watering so I attempted a resurrection by using Scott's Champion fertilizer, Scott's Professional S.T.E.M. (micro-nutrient formula), and Ca fertilizer (Table A7). These fertilizers were alternated with a day of no water between fertilizing. It is likely that the greenhouses at CSU were too hot (23° C with spikes to 29° C in the daytime and 19° C at night) for the normally cool-climate adapted buffaloberry. While the new fertilizing regime seemed to help, the shrubs were still in such bad health that I decided to encourage winter dormancy in hopes that they would be in better health the following spring after breaking dormancy. On October 2, 2009 I moved the shrubs outside to initiate dormancy while weaning them off of fertilizer. On November 17, 2009, I moved them to the large barn on CSU's Waverly property. The shrubs were covered with clean hay and watered 3 times throughout the winter.

Table A7. Nutrient solution used in the greenhouse study adapted from (Huss-Danell 1978). Both calcium compound were mixed together in a separately to prevent it from precipitating out of solution. This solution was used with 4 different levels of $^{15}NH_4{}^{15}NO_3$, mixed separately (Table A8).

Compound		Concentration (1x)	Units
K_2SO_4	Potassium sulfate	1.600	mM
$^{15}NH_4{}^{15}NO_3$	10% atom (^{15}N) ammonium nitrate	see Table A8	mM
$FeC_{l3}*6H_2O$	Ferric chloride, hexahydrate	17.900	uM
Na_2-EDTA	Sodium EDTA	16.900	uM
$MgSO_4 * 7H_2O$	Magnesium sulfate, heptahydrate	2.000	mM
KH_2PO_4	Potassium phosphate, monobasic	0.169	mM
K_2HPO_4	Potassium phosphate, dibasic	0.833	mM
H_3BO_3	Boric acid	23.100	uM
$MnSO_4 * H_2O$	Manganese sulfate, monohydrate	4.600	uM
$ZnSO_4 * 7H_2O$	Zinc sulfate, heptahydrate	0.800	uM
$CuSO_4 * 5H_2O$	Copper sulfate, pentahydrate	0.300	uM
$NaMoO_4 * 2H_2O$	Sodium molybdate, dihydrate	0.200	uM
$CoSO_4*7H_2O$	Cobalt(II) sulfate heptahydrate	0.500	uM
$CaSO_4 * 2H_2O$	Calcium sulfate, dihydrate	0.600	mM
$CaC_{l2} * 2H_2O$	Calcium chloride, dihydrate	0.500	mM

March 5, 2010 the shrubs were moved back to the PGF at CSU and moved into a newly constructed small bay where I hoped I would be able to better control the temperature. The temperature in this bay was set at 15 degrees C. In order to cool down the bay, a shade curtain would be drawn over the entire bay. Since the cooling shade curtain would negatively impact sunlight on the study, there was also supplemental light above every treatment. I started fertilizing the shrubs with half strength N-free fertilizer (Table A7) on Mondays, Ca fertilizer on Wednesdays, and water alone on Fridays. At this point, out of 1,000 cuttings, only 150 of the propagated cuttings had survived. After two weeks both fertilizers were used at full-strength. On April 12, 2010 I began the study using labeled ^{15}N fertilizer treatments and different shading regimes (see study design below). As outdoor temperatures increased and the greenhouse bay warmed up, the plants began to decline in health and die. I suspected again that the shrubs were being over-watered and decreased the amount of water each shrub received. After 90 days I began the process of harvesting the plants for analysis. Nodules were found on only 2 shrubs out of 40 harvested, so I decided to let the study run longer. I moved the study outside the greenhouse, inoculated with a native soil slurry 3 more times, and pruned the stems to encourage nodulation. The study continued for 9 more weeks until the plants were completed harvested in late September 2010. Of the 310 plants harvested, 47 were the reference species, Woods' rose. Of the 263 buffaloberry plants only 40 individuals had live nodules. I do not think that this lack of nodulation is due to the species ability to associate with *Frankia*, but more because the conditions were not ideal for growth and survival in the greenhouse.

Study Design and Methods

Buffaloberry shrubs were treated with varying levels of shade and N additions to investigate if N_2-fixation is affected by these environmental variables. The study was set-up in a split plot design. There were 5 levels of shade treatments (0%, 30%, 40%, 50%, 70%),

which were implemented using shade cloth hung over custom built structures approximately 3 feet above the shrubs. Under each shade treatment there were 4 N addition treatments levels starting with no N addition and 3 levels of increasing amounts of N (Table A8) using an enriched 10 atom % solution of labeled $^{15}NH_4{}^{15}NO_3$. The 4 N and shading treatments were factorially combined leaving a total of 20 treatments of shade and N additions. The shrubs were treated with their corresponding nutrient solution on Mondays, a calcium solution on Wednesdays, and water alone on Fridays to prevent salt and nutrient build-up. A maintenance nutrient solution (Table A8) with a very small amount of added N was applied 3 times throughout the course of the study to the no N treatment to ensure that the plants did not die. Each shade and nutrient treatment combination had a minimum of 12 buffaloberry replicates and 2 reference shrubs (Wood's rose). Each treatment included an equal number of buffaloberry plants ordered from RMNP and 3-4 individuals that were propagated from cuttings. Every 14 to 16 days throughout the course of the study all treatments were randomized.

Table A8. Levels of $^{15}NH_4{}^{15}NO_3$ used in the nutrient solution (Table A7).

$^{15}NH_4{}^{15}NO_3$ Addition Levels	Concentration (mM)
None	0
Maintenance	0.05
Low	0.2
Medium	0.65
High	1.5

At the end of the study, the acetylene reduction technique was used to measure the N_2-fixation by buffaloberry. While the plants were still intact, the roots were washed completely clean of potting media. Then the root system was cut and separated from the aboveground growth and put into a container for the acetylene reduction assay. In addition to running blanks, the assay was also run on the non-nodulated bufflaloberry roots as well

as the reference shrub (Wood's rose) roots as controls. I incubated the roots for 20 minutes in 10% acetylene and stored 25 ml of the gas produced by the incubated samples in a vacuum sealed glass vial (Labco Exetainers) for future analysis with a gas chromatograph. Once the acetylene reduction assay was completed, the foliage, woody material, roots, and nodules were harvested separately and placed in paper bags. The samples were then oven dried at 55 degrees C to a constant mass.

At this point I decided not to do further analysis on the plant material because the lack of nodulation would not have shown any treatment effects. Had I chosen to continue with the analysis, the dry weight of the foliage, woody material, roots, and nodules would have been determined and the samples would have been ground using a Wiley Mill with a size 20-mesh screen. The propagated cuttings would have been analyzed separately from the RMNP individuals. The foliage samples of 6-9 shrubs per treatment were to have been used for mass spectrometer analysis to determine ^{15}N content in the plant material. A small portion of the ground foliage, woody material, roots, and nodules were also to be analyzed for total N content. I would have determined the N_2-fixation rate per gram dry weight of nodules per plant. The dry weights of the aboveground and root biomass would also have helped to determine the amount of N_2-fixation on a per plant basis. The total N content for each plant was to be calculated from the foliar, woody material, and root analyzes.

APPENDIX 3

Inorganic Nitrogen Analysis

At 46 plots I used ion exchange resin bags to measure plant available N. The resin bags were made with 15 grams of both cation and anion resin in sterile nylon sacks. Four to five bags were buried 10 cm deep in each quadrant of the sampling plot in June 2010. The resin bags were left for 3 months and collected in mid-September 2010. N was extracted from the resin with 2 M KCl and analyzed for NO_3^- and NH_4^+. Relationships between NO_3^-, NH_4^+, and total N and other vegetation, environmental, and soil variables were examined by inspecting pair-wise correlations computed in SAS PROC Corr (Table A8).

Table A8. Pearson correlation coefficients, *P*-values, and number of observations comparing environmental, vegetation, $\delta^{15}N$, and soil data with NO_3^-, NH_4^+, and total inorganic N. The best 15 correlations are shown for each variable. The first row is the correlation coefficient, the second row is Prob > |r| under H_0: Rho=0, and the last row is *n*. Using Dinn-Sidak correction $\alpha' = 0.0034$.

	Best 1	**Best 2**	**Best 3**	**Best 4**	**Best 5**	**Best 6**	**Best 7**
Resin NH_4	Resin NH_4	Resin N Total	Resin NO_3	Total Basal Area [e]	Dead lodgepole (BA)	Relative PAR [c]	Foliar $\delta^{15}N$ buffaloberry
	1	0.991	0.970	0.483	0.387	-0.333	0.316
		<.0001	<.0001	0.001	0.009	0.025	0.035
	45	45	45	45	45	45	45
Resin NO_3	Resin NO_3	Resin N Total	Resin NH_4	Total Basal Area	Relative PAR	Foliar $\delta^{15}N$ buffaloberry	Buffer pH
	1	0.994	0.970	0.487	-0.345	0.340	-0.329
		<.0001	<.0001	0.001	0.020	0.022	0.028
	45	45	45	45	45	45	45
Resin N Total	Resin N Total	Resin NO_3	Resin NH_4	Total Basal Area	Dead lodgepole (BA)	Relative PAR	Foliar $\delta^{15}N$ buffaloberry
	1	0.994	0.991	0.489	0.356	-0.342	0.331
		<.0001	<.0001	0.001	0.016	0.021	0.026
	45	45	45	45	45	45	45

Table A8. Continued

	Best 8	Best 9	Best 10	Best 11	Best 12	Best 13	Best 14	Best 15
Resin NH_4	Buffer pH	Mg (ppm)	Ca (ppm)	Alive lodgepole (BA)	Mineral NO_3 [f]	% Clay	N_2 Fixer Present [d]	Na (ppm)
	-0.292	-0.289	-0.244	0.235	0.218	-0.202	-0.198	-0.196
	0.051	0.054	0.106	0.120	0.150	0.183	0.192	0.197
	45	45	45	45	45	45	45	45
Resin NO_3	Dead lodgepole (BA)	Alive lodgepole (BA)	Mineral NO_3	Mg (ppm)	Ca (ppm)	Alive:Dead [b]	% Clay	Location Code [a]
	0.324	0.309	0.267	-0.261	-0.227	0.199	-0.186	-0.177
	0.030	0.039	0.077	0.084	0.134	0.224	0.221	0.246
	45	45	45	45	45	39	45	45
Resin N Total	Buffer pH	Dead lodgepole (BA)	Mg (ppm)	Mineral NO_3	Ca (ppm)	% Clay	N_2 Fixer Present	% Sand
	-0.314	0.277	-0.276	0.246	-0.237	-0.195	-0.177	0.161
	0.036	0.065	0.067	0.103	0.118	0.199	0.244	0.290
	45	45	45	45	45	45	45	45

[a] Location code = Plots were separated by area into 9 categorical codes.
[b] Alive:Dead = The ratio of alive to dead lodge pole pine as measured by basal area
[c] PAR = Photosynthetically active radiation
[d] N_2 fixer present = Presence/absence of legume species (Nevada pea (*Lathyrus lanszwertii* Kellogg var. *leucanthus* (Rydb.) Dorn), silvery lupine (*Lupinus argenteus* Pursh), and goldenbanner (*Thermopsis divaricarpa* A. Nelson)) and other actinorhizal shrubs (Snowbrush (*Ceanothus velutinus* Douglas ex Hook))
[e] BA = Basal area estimated using a 20 basal area factor glass prism
[f] Mineral NO_3 = Mineral soil NO_3 (ppm)
[g] Resin NH_4 = NH_4 as determined by resin bags
[h] Resin NO_3 = NO_3 as determined by resin bags
[i] Resin N Total = NH_4 plus NO_3 (total inorganic N) as determined by resin bags