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NITROGEN DYNAMICS IN THE SAGEBRUSH STEPPE:  
ANTHROPOGENIC FLUXES AFFECT SHRUB-STEPPE PLANT  
COMMUNITIES

By

Roger Long

A thesis

submitted in partial fulfillment

of the requirements for the degree of

Doctor of Arts in the Department of Biological Sciences

Idaho State University

Summer 2016

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Committee Approval

To the Graduate Faculty:

The members of the committee appointed to examine the dissertation of Roger Benjamin Long find it satisfactory and recommend that it be accepted.

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## Acknowledgements

First, and foremost, I'd like to express my gratitude to Dr. Terry Bowyer, who took in a lost and confused graduate student, and set him on the road to a successful completion of his program. After 2 years of work on the evolution of the bat wing, my previous adviser died suddenly, and without him, I did not have the expertise to finish the project. I came up with an idea for an ecology project and Terry helped me flesh it out and showed me how I could get it done in the one year I had left on my Doctor of Arts fellowship.

My other committee members have been hugely helpful as well. Ken Aho literally wrote the book on R programming and statistics, and his knowledge of multivariate statistics was essential to the project. Bruce Finney introduced me to wonderful world of stable isotopes, and gave me the tools to use stable isotopes to really add some weight to my conclusions.

My labmate, Johanna Thalmann, is the next to the last member of the Large Ungulate Study Team, and has the T-shirt to prove it. Johanna was great company in the lab and provided valuable assistance and office supplies. Speaking of office supplies, John Kie was an invaluable source of all kinds of necessary office supplies, scientific equipment, and other necessities. I can never repay him.

Million Hallimichael and Genevie of the ISU Biogeochemistry Lab showed us how to prepare samples and got us our results in a more than timely fashion. Emma McCorkle in Kitty Lohse's lab provided workspace and equipment for preparing our stable isotope samples. Also in Kitty's lab, Stacy King helped out with a troublesome ID of a bryophyte.

All of the people at Stoller-Gonzales (now Western Advantage) were very helpful, especially Jeremy Shive and Roger Blew. Jeremy Shive spent a day with me, showing me around the INL, and gave me the information on the ecological history of the INL that enabled me to choose my study sites. Roger Blew supplied me with all kinds of cool electronics to keep me safe and in touch while we were doing our field work.

I'm also grateful to Stoller-Gonzales for introducing me to Kristin Kaser, who became my intern after she finished her summer fieldwork on the INL. Kristin got me straightened out on my identification of my plant samples, and then went on to create the GIS maps that incorporated the project data into the study areas. In addition, Kristin also contributed to my office supplies, and was a very worthy member of the Large Ungulate Study Team.

I'm very grateful for the time I got to spend working with my previous advisor, Chris Cretekos, Chris was one of the smartest people I ever knew. Thanks also to Richard Behringer, who reminded me that I am really an ecologist at heart, and put me on the path

to starting and finishing this project. Mike Thomas provided me with lab space, and some good advice in the aftermath of Chris's death.

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## ABSTRACT

We sampled vegetation cover, biomass, and diversity on two ostensibly similar sagebrush-steppe sites at the Idaho Nuclear Laboratory in east-central Idaho, USA, to elucidate effects of livestock grazing on cold desert plant communities, and possible effects of nutrient transfer from adjacent agricultural fields. We sampled 107 quadrats on two sites, one currently grazed and one that has not been grazed by livestock in ~70 years. We determined that plant cover, biomass, and diversity were all higher on the ungrazed site, although species richness was greater on the grazed site. We used stable isotope analysis of plant tissues to identify nitrogen isotopic signatures of sage-steppe plant species, and to determine constraints to nitrogen availability for herbivores in ecosystems adjacent to agricultural fields. We sampled vegetation for analysis at two ostensibly similar sagebrush-steppe sites adjacent to agricultural (alfalfa and wheat) fields in Central Idaho, USA, with different grazing regimes: one ungrazed by livestock for ~70 years; and one with historical and current grazing. Three shrub species, big sagebrush (*Artemisia tridentata*), dwarf goldenbush (*Ericameria nana*), and winterfat (*Krascheninnikovia lanata*); and two grasses, Indian ricegrass, (*Achnatherium hymenoides*) and squirreltail grass (*Elymus elymoides*), were sampled at 100-m intervals to a distance of 2 km from the edge of agricultural sites. Our findings were that the grazed site was more nitrogen limited than the ungrazed site, and plants occurring at greater distances from agricultural fields also were increasingly nitrogen limited. We used Principal Components Analysis (PCA) to determine the contributions of the <sup>15</sup>N and C:N characteristics of plant species under consideration in distinguishing sites and

distances from fields. In those analyses, we observed that  $^{15}\text{N}$  was strongly associated with site (grazed or ungrazed) and, to a lesser degree distance from agricultural fields, whereas C:N was more associated with distance than site. Based on these results, we hypothesize that the differences in plant communities we observed between the two sites is the result of nitrogen export from the grazed site in the form of livestock biomass.

## CHAPTER 1

### EFFECTS OF AGRICULTURE AND LIVESTOCK: ANTHROPOGENIC NITROGEN FLUXES IN THE SHRUB-STEPPE.

#### **Abstract**

We used stable isotope analysis of plant tissues to identify nitrogen isotopic signatures of sage-steppe plant species, and to determine constraints to nitrogen availability for herbivores in ecosystems adjacent to agricultural fields. We sampled vegetation for analysis at two ostensibly similar sagebrush-steppe sites adjacent to agricultural (alfalfa and wheat) fields in Central Idaho, USA, with different grazing regimes: one ungrazed by livestock for ~70 years; and one with historical and current grazing. Three shrub species, big sagebrush (*Artemisia tridentata*), dwarf goldenbush (*Ericameria nana*), and winterfat (*Krascheninnikovia lanata*); and two grasses, Indian ricegrass, (*Achnatherium hymenoides*) and squirreltail grass (*Elymus elymoides*), were sampled at 100-m intervals to a distance of 2 km from the edge of agricultural sites. Our findings were that the grazed site was more nitrogen limited than the ungrazed site, and plants occurring at greater distances from agricultural fields also were increasingly nitrogen limited. We used Principal Components Analysis (PCA) to determine the contributions of the  $^{15}\text{N}$  and C:N characteristics of plant species under consideration in distinguishing sites and distances from fields. In those analyses, we observed that  $^{15}\text{N}$  was strongly associated with site (grazed or ungrazed) and, to a lesser degree distance from agricultural fields, whereas C:N was more associated with distance than site. ANOVAs and Inverse Distance Weighting (IDW) maps support those interpretations, and confirm the

importance of site in explaining the difference in C:N between grazed and ungrazed areas. Those maps also illustrate the patchy nature of the distribution of  $^{15}\text{N}$  and C:N in the study areas.

## **1. Introduction**

### *1.1 Nitrogen dynamics of sagebrush-steppe*

In the 21<sup>st</sup> century, discussion of sagebrush-steppe often includes terms such as degraded (Sands et al., 1999), deteriorating (Knick, 1999), declining (Siegel Thines et al., 2004), and disappearing (Knick et al., 2003). Sagebrush-steppe is the largest semi-arid ecosystem in North America; however, few stands remain in their original state (Fleischner, 1994; Rowland et al., 2006). Much of the sagebrush-steppe currently is used for livestock grazing, and considerable areas can be described as overgrazed (Fleischner 1994; Anderson and Inouye 2001). Invasion of exotic annual grasses (West, 2000), fire (D'Antonio and Vitousek, 1992), agriculture (Brandt and Rickard, 1994), and development for oil and gas extraction (Avirmed et al., 2014) also have damaged large areas of sagebrush-steppe, to the extent that the area currently dominated by big sagebrush (*Artemisia tridentata*) has been reduced to ~17.5 million ha, less than one-half of what was present at the time of European settlement (Rowland et al., 2006). Even when stocking rates of domestic grazing animals have been reduced, many areas have failed to recover, and may now represent new equilibria, characterized by reduced populations of forbs and deep-rooted shrubs (West et al., 1984). The inability of those areas to return to their original state may relate to the disruption of nutrient cycles, especially of nitrogen (Evans and Ehleringer, 1993).

Current threats to sagebrush-steppe ecosystems underscore the need to understand nitrogen cycling, N fluxes, and especially effects of anthropogenic influences on N in the shrub-steppe environment. Potential anthropogenic factors influencing N fluxes include damage to macrobiotic crusts from by trampling by livestock (Bowker et al., 2005), export of N in the form of cattle biomass, inputs of N from neighboring agricultural fields, and atmospheric deposition. The objective of our study is to examine effects of adjacent agricultural fields and livestock grazing on nitrogen dynamics of plant species that characterize sagebrush-steppe plant communities. Anthropogenic supplementation of N may dramatically alter sagebrush-steppe plant communities (Evans and Belnap, 1999) with concomitant effects on native herbivores, including smaller, often threatened vertebrates, such as greater sage grouse (*Centrocercus urophasianus*), pygmy rabbits (*Brachylagus idahoensis*) and more abundant jackrabbits (*Lepus* spp.) with high metabolic rates, which require diets lower in fiber and higher in N (Karasov, 1990; Kleiber, 1961; McNab, 2002).

### *1.2 Nitrogen isotopic fractionation*

Nitrogen fixed by symbionts such as *Rhizobium*, and nitrogen fertilizer created by the Faber-Bosch process has the same  $\delta^{15}\text{N}$  signature as  $\text{N}_2$  in the atmosphere (i.e.,  $\delta^{15}\text{N} = 0\text{‰}$ ). Variability of  $\delta^{15}\text{N}$  in plant tissues can occur because of discrimination towards  $^{14}\text{N}$  in uptake. This outcome occurs because the heavier isotope,  $^{15}\text{N}$ , is chemically less active, and creates stronger chemical bonds than the lighter isotope (Ben-David and Flaherty, 2012; Peterson and Fry, 1987). When the availability of N is limited compared

with its demand, less discrimination against the less biologically active  $^{15}\text{N}$  occurs, and plants will have higher levels of  $\delta^{15}\text{N}$  (Ben-David and Flaherty, 2012; Dawson et al., 2002). Consequently,  $\delta^{15}\text{N}$  in plant tissues can be an indicator of lower N availability for those plants. Additionally, plants tend to accumulate  $^{15}\text{N}$  as they age, or become more water stressed. Deep-rooted plants that uptake N from deeper soil layers also tend to have higher  $\delta^{15}\text{N}$  (Dawson et al., 2002).  $^{15}\text{N}$  accumulates in deeper soil layers because of denitrification and de-ammonification volatilization processes that favor the more active  $^{14}\text{N}$ , leaving  $^{15}\text{N}$  behind (Dawson et al., 2002).

We hypothesized that differences in plant tissue  $\delta^{15}\text{N}$  and C:N would manifest between sites with and without cattle grazing, and within sites at varying distances from adjacent nitrogen-enriched agricultural fields in sagebrush-steppe ecosystems in southeastern Idaho. Agricultural fields often leach nitrogen to bordering ecosystems as a result of wind and hydrologic processes, or via deposition by native herbivores feeding in agronomic sites and defecating and urinating in adjacent lands (Stewart et al., 2010). Thus, we predict that there is ongoing transfer of nitrogen from the agricultural fields into the shrub-steppe.

Our specific predictions are that effects of grazing on sage-steppe  $\delta^{15}\text{N}$  will be greatest in shorter-lived, shallower-rooted plants, because limitations to N availability at grazed sites will cause plants to uptake more  $^{15}\text{N}$ . Conversely, longer-lived, deep-rooted plants will have consistently higher  $\delta^{15}\text{N}$ , and show fewer differences between the grazed and ungrazed sites. Additionally, we predict that samples collected from plots at greater

distances from agriculture will be enriched in  $^{15}\text{N}$  compared with those taken from plots adjacent to agricultural fields, because greater N availability near the agricultural fields will allow greater fractionation of N. We also predict that C:N will be greater in samples taken from grazed sites, that C:N will increase along a gradient from the samples proximal to agricultural fields to distant samples, and that a positive correlation will occur for C:N and  $\delta^{15}\text{N}$ , because high values of both  $\delta^{15}\text{N}$  and C:N indicate N limitation (Dawson et al., 2002). To properly test these predictions, we required that our study sites be large enough to be representative of the shrub-steppe ecosystem, and that we had sufficient replication within those sites to obtain adequate sampling size.

Studies using stable isotopes to examine N cycling in shrub-steppe ecosystems often focus on soil processes such as mineralization, deammonification and denitrification. Much soil N, however, exists in forms or locations not readily available to plants (Binkley and Hart, 1989), and plants have been demonstrated to uptake organic N, both with and without the assistance of mycorrhizae (Dawson et al., 2002). We were interested in N as a characteristic reflecting plant interactions with the environment and concomitant effects on herbivores. Thus, our focus was on N in plants rather than soils.

## **2. Methods**

### *2.1 Study Site*

The study area was situated on the Idaho National Laboratory (INL) in southeastern Idaho, USA (Fig. 1). We considered two ostensibly similar sagebrush-steppe sites; one located near the terminus of Birch Creek (Fig. 1), and the other near the town of Howe (Fig 1). Each study site measured approximately  $2 \times 3$  km (Fig. 1). We believed that

those large study sites were necessary to ensure that the sites would be typical of the sagebrush-steppe ecosystem, and would also allow for adequate replication within sites. We assumed that those sites are typical of grazed and ungrazed areas in the shrub-steppe ecosystem. Sites that have not been grazed by livestock for >20 years are difficult to find. Vegetation on the study site was cold desert shrub-steppe, characterized by big sagebrush (Anderson and Inouye, 2001). Topography at both sites is flat with minimal effects of aspect. Annual precipitation (from 2007 to 2015) at Howe and Birch Creek (Blue Dome) were 15.7 cm and 16.4 cm, respectively (NOAA). The site near Howe is currently grazed at 13.8 Animal Unit Months (AUM) (personal communication, J. Smith, Bureau of Land Management, 2016). An AUM is the amount of forage needed by a cow-calf pair for 1 month. This value is usually calculated at one AUM = 12 kg of forage per day. The site near Birch Creek has not been grazed since the INL was established in 1947 (Personal communication, J. Shive, Western Advantage, 2015). Grazing by native herbivores was sparse on both sites. Those herbivores included North American elk (*Cervus elaphus*), mule deer (*Odocoileus hemionus*), pronghorn (*Antilocapra americana*), black-tailed jackrabbits (*Lepus californicus*), greater sage grouse, and other smaller herbivores. Data on snow accumulation for the Howe and Blue Dome weather stations was not available, but snowfall at the INL Central Facility averaged 38 cm annually. The major soil type at Howe is Sparmo silt loam, which is well drained with an A soil horizon averaging 23 cm deep. The main soil type at Birch Creek is Whiteknob gravelly loam, which is excessively well drained, and has an A soil horizon averaging 12.5 cm deep. Both soil types are considered prime farmland when irrigated (<http://www.casoilresource.lawr.ucdavis.edu/gma>). There are approximately 115 frost-

free days per year at both sites

(<http://www.cals.uidaho.edu/edcomm/pdf/bul/bul0857.pdf>). The growing season usually extends from mid-April to late September.

Both sites are bordered by agricultural fields. The grazed site (Howe) is bordered on the north by both wheat (*Triticum aestivum*) and alfalfa (*Medicago sativa*) fields, and on the west by alfalfa fields. There is no surface water near the grazed site; consequently, water is brought in by truck for grazing livestock to a point approximately 2 km SE of the SE corner of that study site. The ungrazed site (Birch Creek) is bordered on the east by wheat and alfalfa fields, and no supplemental sources of water are available; ranchers are not allowed to transport water into this area. Birch Creek terminates in a series of irrigation canals approximately 1.5 km north of the ungrazed study site. At both sites, wheat fields are fertilized with both nitrogen (N) and phosphorus (P), whereas alfalfa, a nitrogen-fixing legume, is fertilized only with phosphorus.

## *2.2 Sampling Methods*

Sampling at both sites was conducted during summer of 2015. Both sites were divided into 10 intervals, at increasing 100-m increments from an agricultural border with an 11<sup>th</sup> interval placed 2 km from the agricultural field. Five 5 x 5 m quadrats were placed randomly within each 100-m interval, for a total of 55 replicate sampling quadrats on each study site. For purposes of analyses, we combined the 11 distance categories into three distances: 0-300 m; 300-600 m; and 600-1,000 m. Three equidistant transect lines

were placed across each quadrat. The direction in which transects were sampled (N-S or E-W) was decided by a coin flip. Cover for each plant species was measured with the line-intercept method Along transects (Kaiser, 1983). If a gap of >5 cm occurred in an individual plant along the transect line, the distance across the gap was omitted from the calculation of cover. For plants other than shrubs, all plant tissues that intersected a transect line were harvested by clipping from ground level upward. For each shrub intersecting the vertical projection of a transect line, 25 leaders of new growth were clipped. Plant samples were dried at 50° C until weight remained constant for 24 h. Samples were weighed to the nearest 0.1 g on a Fisher Scientific Industries SLF501-US scale (Pittsburgh, Pennsylvania, USA).

Five species, two grasses and three shrubs, were deemed sufficiently abundant across both study sites and sampling intervals for valid analyses of differences between sites and among distances for  $\delta^{15}\text{N}$  and C:N from agricultural areas. Grass species were Indian ricegrass (*Achnatherum hymenoides*) and squirreltail grass (*Elymus elymoides*). Shrubs included big sagebrush, dwarf goldenbush (*Ericameria nana*), and winterfat (*Krascheninnikovia lanata*).

### 2.3 Data Analyses

We tested for differences in  $\delta^{15}\text{N}$  and C:N between study sites and among distance categories using two-way ANOVAs with site and distance from agricultural fields as main effects. All statistical analyses were performed in R version 3.02 (R core team 2014, Aho, 2013). One outlying value was omitted in analyses for winterfat. All data

were examined with Shapiro-Wilk tests for normality, and Fligler-Killeen tests for heteroscedasticity. Assumptions for homoscedasticity for C:N for *A. hymenoides* were not met and were not correctable using transformations, prompting the use of the Brunner-Dette-Munk test (Brunner et al. 1997), which allows testing of interactions in two-way designs, given violations of normality and constant variances. We used  $\alpha = 0.05$ . To allow multivariate consideration of the characteristics of plant species at sampling locations, we conducted Principal Components Analysis (PCA). This application used correlation matrices of  $\delta^{15}\text{N}$  and C:N for the five plant species (Gardener, 2014). We used vector analysis (Oksanen et al., 2015) to interpret results.

Inverse Distance Weighting (IDW) was used to provide bivariate representations of variation in  $\delta^{15}\text{N}$  composition and C:N of plants at two sites and across distances from agricultural lands. IDW interpolates heteroscedastic data without the need for transformation, while maintaining spatial interpolation accuracy (Isaaks et al. 1989, Cooke et al., 1993; Weber and Englund, 1992). IDW was more appropriate than the stochastic method of kriging, because kriging requires assumptions of stationarity and isotropy (Cressie, 1985). Additionally, an insufficient sample size was available to de-trend data for universal kriging, which assumes a trend in data (Peterson et al., 2013; Li and Heap 2014). We used Moran's I (Bolstad 2012, Sawada 2015) to identify the spatial character of data (e.g., clustered, random, or dispersed at different spatial scales). We used a High-Low Clustering Getis-Ord General G tool to examine specific clustering characteristics. All IDW analyses were performed within ArcMap 10.3.3 (ESRI, Redlands, California, USA).

## 2.4 Isotope analyses

Samples from plant species collected in the summer 2015 were dried, ground to powder, and analyzed with standard methods (e.g., Dawson et al., 2002) at the Idaho State University Interdisciplinary Laboratory for Elemental and Isotopic Analysis with a Costech ECS 4010 elemental analyzer interfaced to a Thermo Delta V Advantage continuous-flow isotope-ratio mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA). Analytical precision, calculated from analysis of standards distributed throughout each analysis, was  $\leq 0.2$  ‰. Isotopic values are reported in the conventional  $\delta$ -notation ( $\delta = ([R_{\text{sample}}/R_{\text{standard}}] - 1) \times 1000$ , where  $R = {}^{15}\text{N}/{}^{14}\text{N}$ ) relative to the international standard atmospheric nitrogen expressed as per mil (‰) (Ben-David and Flaherty, 2012).

## 3. Results

We sought to quantify the influence of cattle grazing and distance from agricultural fields on nitrogen characteristics of five sagebrush-steppe plant species. We documented that means for  $\delta^{15}\text{N}$  were higher, for all five species, in the grazed site (Howe) than on the ungrazed site (Birch Creek) (Table 1). Values for  $\delta^{15}\text{N}$  were significantly higher for all plants combined on the grazed site than on the ungrazed site ( $F_{1,104} = 5.71$ ,  $p < 0.02$ ). Additionally, values of  $\delta^{15}\text{N}$  increased with distance categories from the agricultural fields for all plants, but the p-value was only weakly significant ( $F_{2,104} = 3.02$ ,  $p = 0.05$ ). Values for  $\delta^{15}\text{N}$  for the two grass species on the grazed site (Table 1) were significantly

higher ( $F_{1,35} = 28.01$ ,  $p < 0.0001$ ) than on the ungrazed site. Grasses also differed significantly by distance from agriculture ( $F_{2,35} = 8.53$ ,  $p < 0.001$ ). The three shrub species had somewhat higher  $\delta^{15}\text{N}$  values on the grazed site, however, the difference was not significant ( $F_{1,46} = 0.69$ ,  $p > 0.40$ ). Shrubs also did not differ significantly for  $\delta^{15}\text{N}$  by distance category ( $F_{2,46} = 0.72$ ,  $p > 0.49$ ). C:N values differed significantly for all plants by distance category ( $F_{2,104} = 3.97$ ,  $p < 0.02$ ), but did not differ significantly for all plants combined by site ( $F_{1,104} = 1.40$ ,  $p > 0.23$ ). C:N also differed significantly for both grasses ( $F_{2,35} = 6.78$ ,  $p < 0.004$ ) and shrubs ( $F_{2,35} = 4.09$ ,  $p < 0.03$ ) by distance category, but not by site ( $F_{1,35} = 0.54$ ,  $p > 0.40$ ) or distance ( $F_{1,46} = 2.09$ ,  $p > 0.16$ ).

In a Principal Component Analysis examining plant  $\delta^{15}\text{N}$ , the first principal component represented a grazing axis with ungrazed (negative loadings) vs. grazed sites (positive loadings) and explained 73% of the variation (Fig. 2). The second principal component contrasted plant growth forms with respect to shrubs (negative loadings) and grasses (positive loadings), and explained an additional 15% of the variation. The overlaid arrows in Fig. 2 indicate the direction of most rapid increase in  $\delta^{15}\text{N}$  for each plant species, whereas the length of arrows indicates the strength of correlation of those values with the displayed principal components (Fig. 2). The third principal component can be explained as a “distance from field” axis, extending from near (negative loadings) to far (positive loadings) from agriculture, and explained an additional 8% of the variation. In total, the first three PCs explained a total of 96% of the total variation in tissue  $\delta^{15}\text{N}$  for the five species.

We also used PCA to consider plant C:N characteristics (Fig. 3). In this instance, PC 1 explained 58% of the variation, with values ranging from near (negative loadings) to far (positive loadings) from agriculture. PC 2 explained 28% of the variation ranging from the ungrazed site (negative loadings) to the grazed site (positive loadings). PC 3 explained 13% of the variation in the analysis, and contrasted sampling units with high shrub C:N ratios (negative loadings) from those with high grass C:N ratios (positive loadings). In total, the first three axes of the PCA explained 98% of the variation in C:N.

A Spearman rank correlation, which can be used to consider monotonic but nonlinear associations, indicated that  $\delta^{15}\text{N}$  was negatively correlated with C:N ( $r_s = -0.221$ ,  $p = 0.02$ ). Notably, this relationship was strongly driven by the presence of winterfat, which has low C:N values and high  $\delta^{15}\text{N}$  values (Table 1) compared with the other plants in the study. Winterfat may not discriminate against  $^{15}\text{N}$  when taking up soil nitrogen. The Inverse Distance Weighting (IDW) maps also illustrate this relationship; the NW corner of the grazed site shows high  $\delta^{15}\text{N}$  and low C:N, because of a high incidence of winterfat in samples at that location (Fig. 4). Analogously, the IDW maps demonstrate a gradient of  $^{15}\text{N}$  on the ungrazed site with low  $\delta^{15}\text{N}$  values adjacent to the agricultural fields and increasing values in more distant samples. The IDW map of C:N illustrated the low C:N concentration in the SW corner corresponding to the high incidence of winterfat at that location (Fig. 5). Our data were significantly clustered ( $p < 0.01$ ) for  $\delta^{15}\text{N}$  on the grazed location and for C:N at the ungrazed location (Birch Creek). We determined that  $^{15}\text{N}$  and C:N clusters occurred randomly at our sites.

## 4. Discussion

### 4.1 Tests of Hypotheses

When considering all plant samples, the Howe and Birch Creek site differed significantly with respect to tissue  $\delta^{15}\text{N}$ . This outcome occurred primarily because of large differences in  $\delta^{15}\text{N}$  of grasses, particularly *A. hymenoides*, between the two sites, indicating the possibility of greater N limitation in shallower soil horizons on the grazed site (Howe). In contrast, the lack of significant differences in  $\delta^{15}\text{N}$  for shrubs between the two sites may be attributed to the greater age of the shrubs and the deeper soil horizons from which their roots draw nitrogen (Peterson and Fry, 1987). Plant tissue  $\delta^{15}\text{N}$  and C:N also varied with distance from agricultural fields for both grass species under consideration, with lower levels of C:N and  $\delta^{15}\text{N}$  nearer agricultural fields. This result supports our hypothesis that nitrogen is being transferred from the agricultural fields into the native vegetation.

There are three possible mechanisms for explaining nutrient transfer from agricultural fields: a) nutrients in dust could be blowing into the native vegetation on prevailing winds; b) hydrologic processes including runoff could transfer nutrients; and c) native herbivores could transfer nutrients from the agricultural fields to the shrub-steppe. We posit that the most likely mechanism for this transfer of N is native herbivores that live primarily in the shrub-steppe and feed in the adjacent nitrogen-enriched agricultural fields. Native herbivores move between the native shrub-steppe and agricultural fields adjacent to shrub-steppe (Stewart et al., 2010), and feed in the nitrogen-enriched fields of

wheat and alfalfa. The two other mechanisms, wind and runoff, are unlikely causes of N-export; prevailing wind on the INL is from the SW, whereas the grazed site is south of the agriculture and the ungrazed site is west of the agriculture. Runoff is also unlikely to contribute to N-transport on our study sites. The terrain of the is flat and soils are coarse, well-drained to excessively well-drained, and there are no signs of runoff erosion at either site.

Effects of nitrogen transfer from agriculture are more pronounced in shallower soil horizons as demonstrated by the lower C:N and  $\delta^{15}\text{N}$  in shallow-rooted plants sampled adjacent to agricultural fields. A significant difference occurred in C:N for all plants combined between the two sites, mainly because of the extremely large effect of one species of grass, *A. hymenoides*. There was a negative correlation between  $\delta^{15}\text{N}$  and C:N over all plants combined, mostly because of effects of winterfat, which had higher  $\delta^{15}\text{N}$  and lower C:N than other study plants. When winterfat was removed from the correlation, however, no significant relationship occurred between C:N and  $\delta^{15}\text{N}$ .

Winterfat's relatively high tissue  $\delta^{15}\text{N}$ , indicates that this species, unlike most other plant species (Evans, 2001; Peterson and Fry, 1987) may not strongly discriminate between  $^{14}\text{N}$  and  $^{15}\text{N}$ . Winterfat was also the only species that exhibited no significant association between N-measures ( $\delta^{15}\text{N}$  and C:N) and explanatory variables (site and distance from agricultural fields) in our study. *K. lanata* had the deepest roots of any of the plants in the study, but all winterfat plants sampled, regardless of age or size, had a similar  $\delta^{15}\text{N}$  signature (Table 1). Moreover, winterfat has a symbiotic relationship with vesicular-arbuscular mycorrhizae (Snyder and Friese, 2014).

Principal component analyses indicated that site was the strongest correlate of  $\delta^{15}\text{N}$ , with distance from agriculture playing an important but lesser role. Conversely, for C:N, distance from agriculture was the most important variable, followed by site. The Inverse Distance Weighting maps for  $^{15}\text{N}$  also indicated that site is correlated with variation in  $\delta^{15}\text{N}$ , but that distance from agriculture played a major role on the ungrazed site. The IDW maps for C:N indicated lateral gradients of C:N on both sites. This effect may result from the influence of agricultural fields located 1 km north of the ungrazed site and 300 m west of the grazed site. In addition, although C:N was not significantly different for all plants across sites, that ratio did explain ~40% of the variation in C:N; the IDW maps show a definite difference with the ungrazed site having much greater areas of low C:N. The IDW maps also show the patchy nature of the  $\delta^{15}\text{N}$  and C:N distributions, which would not have been possible with inferential statistics alone.

We had difficulty finding sufficient samples of the same species on both sites at the same distances from agricultural fields. Although we did obtain enough samples of the three most common shrubs and the two most common grasses, we were unable to obtain sufficient samples of forb species for comparison. Other studies have shown forbs often do not recover from disturbance even after native grasses and shrubs have become re-established (Avirmed et al., 2015).

When paired with more traditional elemental analyses, stable isotopes can provide insights concerning anthropogenic-related N-fluxes in the shrub-steppe (Ehleringer et al.,

1998). We were most interested in using stable isotope analysis to detect possible N limitation as indicated by decreasing discrimination against  $^{15}\text{N}$ . Plants under N limitation in sage-steppe have increased levels of  $\delta^{15}\text{N}$  and higher C:N in leaf tissues than conspecifics not experiencing limitation (Ehleringer et al., 1998). Discrimination against  $^{15}\text{N}$  occurs during enzyme-mediated reactions during N uptake by mycorrhizae and plant roots. The greater the proportion of the soil N pool that is converted to plant tissues, the more the plant tissues will equilibrate to the source  $\delta^{15}\text{N}$  (Dawson et al., 2002). Values for soil  $\delta^{15}\text{N}$  in shrub-steppe soils average 5-7‰ (Ehleringer et al., 1998).

As predicted, our study demonstrated lesser effects of grazing and distance from agriculture on deep-rooted shrubs as opposed to shallow-rooted grasses. For instance, C:N and  $\delta^{15}\text{N}$  did not vary significantly for winterfat or big sagebrush by either site or distance category, whereas Indian ricegrass showed significant differences for C:N and  $\delta^{15}\text{N}$  across both site and distance, as did squirreltail grass. These results lend support to previous work indicating that big sagebrush and other deep-rooted shrubs increase nitrogen in upper soil horizons by taking nitrogen up from deep soil layers and depositing it as leaf litter and fine root structures (Cardon et al., 2013). Our finding that deep-rooted shrubs have lower C:N and higher  $\delta^{15}\text{N}$  across both sites indicates that those shrubs are drawing nitrogen from deeper soil horizons, and that those deeper soil horizons are not as strongly affected by effluxes such as livestock grazing, at least at the levels of grazing on our study site. Several studies have documented changes in nitrogen cycling when shrub-steppe communities are replaced by shallow-rooted exotic annual grasses (Evans et al., 2001; Rimer and Evans, 2006; Sperry et al., 2006). Our study indicates that much of the

nitrogen in the shrub-steppe is stored in deep soil horizons, which would be unavailable to plants without deep-rooted shrubs.

Research that examines N effluxes from cattle grazing tend to focus on effects on cryptobiotic crusts (Dobkin and Sauder, 2004; Evans and Ehleringer, 1993; Fleischner, 1994). Evans and Ehleringer, (1993) demonstrated that cryptobiotic crusts were the primary nitrogen sources in shrub-steppe on the Colorado Plateau, and that disturbance of those crusts caused a net loss of nitrogen from the ecosystem and elevated  $\delta^{15}\text{N}$ . Another possible cause of differences we detected is export of nitrogen from the shrub-steppe ecosystem in the form of livestock biomass. For every 100 kg of mass gained by cattle grazing on the shrub-steppe, nitrogen composes approximately 3 kg (Parmenter and MacMahon, 2009). This results in a net export of nitrogen that is not replaced, even though some supplemental feeding of cattle may occur.

Selective grazing of plants with lower lignin and cellulose and more leaf nitrogen (lower C:N) can make significant differences in body fat, age at first reproduction, bodyweight of young, and milk production in ungulates such as elk, mule deer, and pronghorn (White, 1983). The C:N would likely have even larger effects on smaller herbivores such as sage grouse and jackrabbits because of their higher rate of metabolism (Kleiber, 1961), and lower gut capacity (Demment and Van Soest, 1985).

## *4.2 Conclusions*

Our results support our predictions that grazed sites would have higher values for  $\delta^{15}\text{N}$  and C:N for shallow-rooted, short-lived grasses, indicating greater N limitation. Our study indicates that animals feeding in nitrogen-enriched agricultural fields likely transfer nitrogen to native sage-steppe. The C:N data reflect nitrogen content in the plant tissues, whereas the  $\delta^{15}\text{N}$  data provide information on sources of influxes and effluxes of N to the shrub-steppe. Future directions for research should include studies of plant cover, biomass and diversity of shrub-steppe adjacent to agriculture, and grazed vs. ungrazed sites, to better understand how those factors relate to N fluxes. Moreover, the importance of nutrient export in the form of livestock biomass as needs to be examined in relation to biomass, plant cover, and biodiversity. Paired with stable isotope techniques, these studies can elucidate effects of anthropogenic N fluxes on plant nitrogen pools available to wildlife consumers. Our study was unique among studies of this nature because our study sites were comparatively large—6 km<sup>2</sup>—and that we sampled 55 quadrats of 25 m<sup>2</sup> from each study site. This detailed and fine-scale analysis allowed us to examine the patchy nature of N fluxes in sage-steppe. Our sampling design also allowed us to examine two anthropogenic nitrogen fluxes with differing effects on nitrogen dynamics in an imperiled ecosystem.

## **Acknowledgements**

Funding was provided by a grant from the College of Science and Engineering at Idaho State University. Mariah Blackhorse assisted with sampling and other field work. Dr. Kathleen Lohse and Emma McCorkle provided workspace in the Center for Ecological Research at Idaho State University. Million Hallimichael and the crew in the

BioGeochemistry Lab at ISU helped with sample preparation and ran samples. Dr. Sarah Godsey assisted with the IDW maps. Johanna Thalmann provided invaluable assistance in resolving Microsoft office problems.

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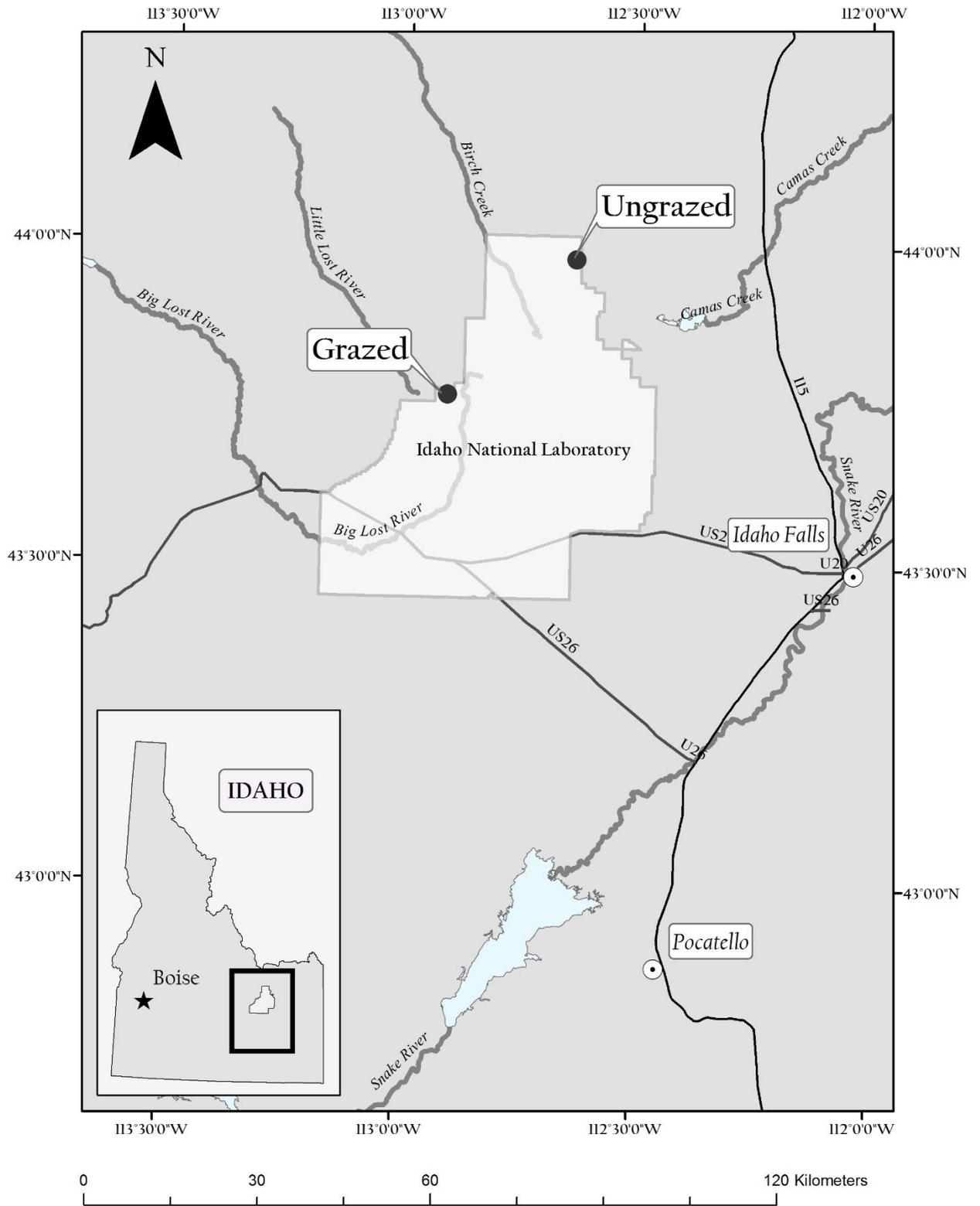


Figure 1. Extant map of study-site locations with map inset of Idaho, USA. The Big Lost River and Birch Creek no longer flow within the boundaries of the INL, because of irrigation withdrawals. As a result, for most of the year, there is no natural surface water on the Idaho National Laboratory (INL).

Table 1. Summary Statistics for  $\delta^{15}\text{N}$  and C:N for the grazed (Howe) and ungrazed (Birch Creek) sites, southeastern Idaho, USA, during summer 2015.

<b>Birch Creek</b>				
<b><math>\delta^{15}\text{N}</math></b>				
	$\bar{X}$	SD	Range	$\bar{X}$
A. <i>hymenoides</i>	-0.43556	1.78252	-4.11	1.99
<i>Elymus</i> spp.	-0.599	1.192891	-2.18	1.1
A. <i>tridentata</i>	1.502308	1.659228	-0.46	4.89
<i>E. nana</i>	1.034286	1.117344	-0.87	2.46
<i>K. lanata</i>	7.0325	1.200211	5.63	8.98
<b>Birch Creek</b>				
<b>C:N</b>				
	$\bar{X}$	SD	Range	$\bar{X}$
A. <i>hymenoides</i>	24.665	4.358395	18.7619	33.218
<i>Elymus</i> spp.	28.24997	5.823694	19.3462	37.075
A. <i>tridentata</i>	23.89737	2.088363	20.1459	27.6891
<i>E. nana</i>	23.65752	3.254509	16.1495	29.6197
<i>K. lanata</i>	20.37374	1.378989	18.3712	22.6927

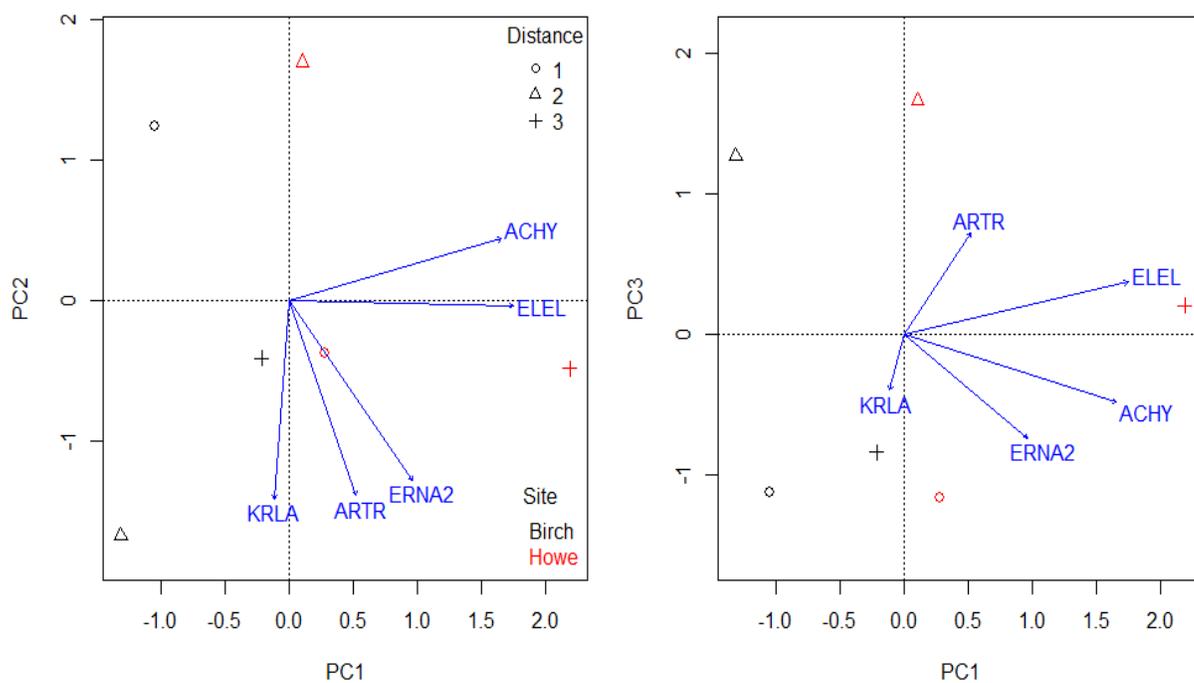


Figure 2. Principal components analysis for  $\delta^{15}\text{N}$ . Axis 1 corresponds best to site, and axis 2 corresponds best with plant growth form. Axis 3 corresponds best with distance from agricultural fields in southeastern Idaho, USA, during summer 2015.

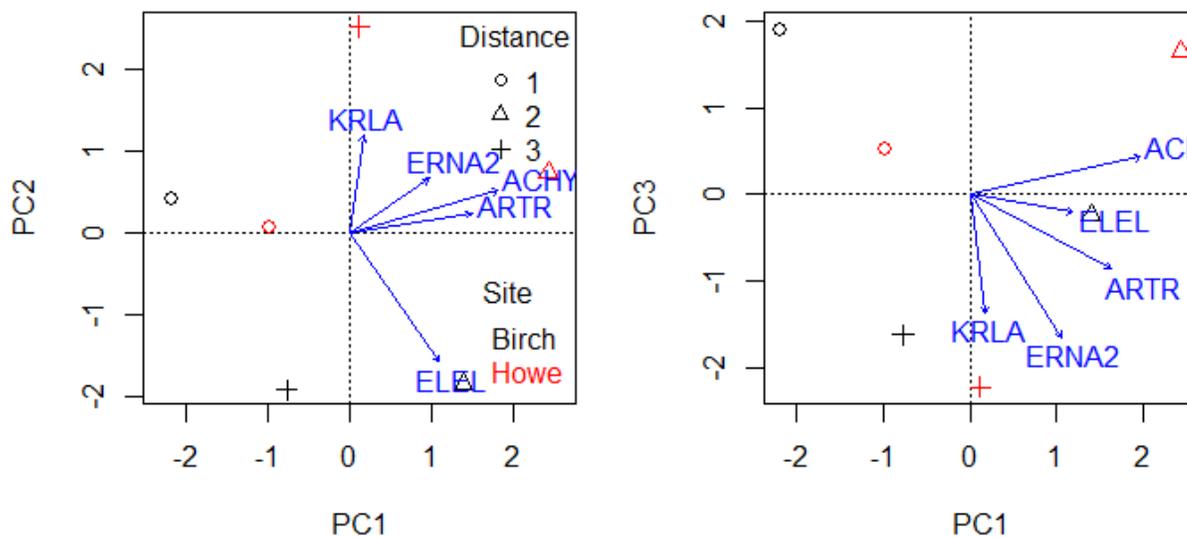


Figure 3. Principal components analysis for C:N. Axis 1 corresponds best to distance from agricultural fields, and axis 2 corresponds best to site. Axis 3 corresponds best to growth form in southeastern Idaho, USA, during summer 2015.

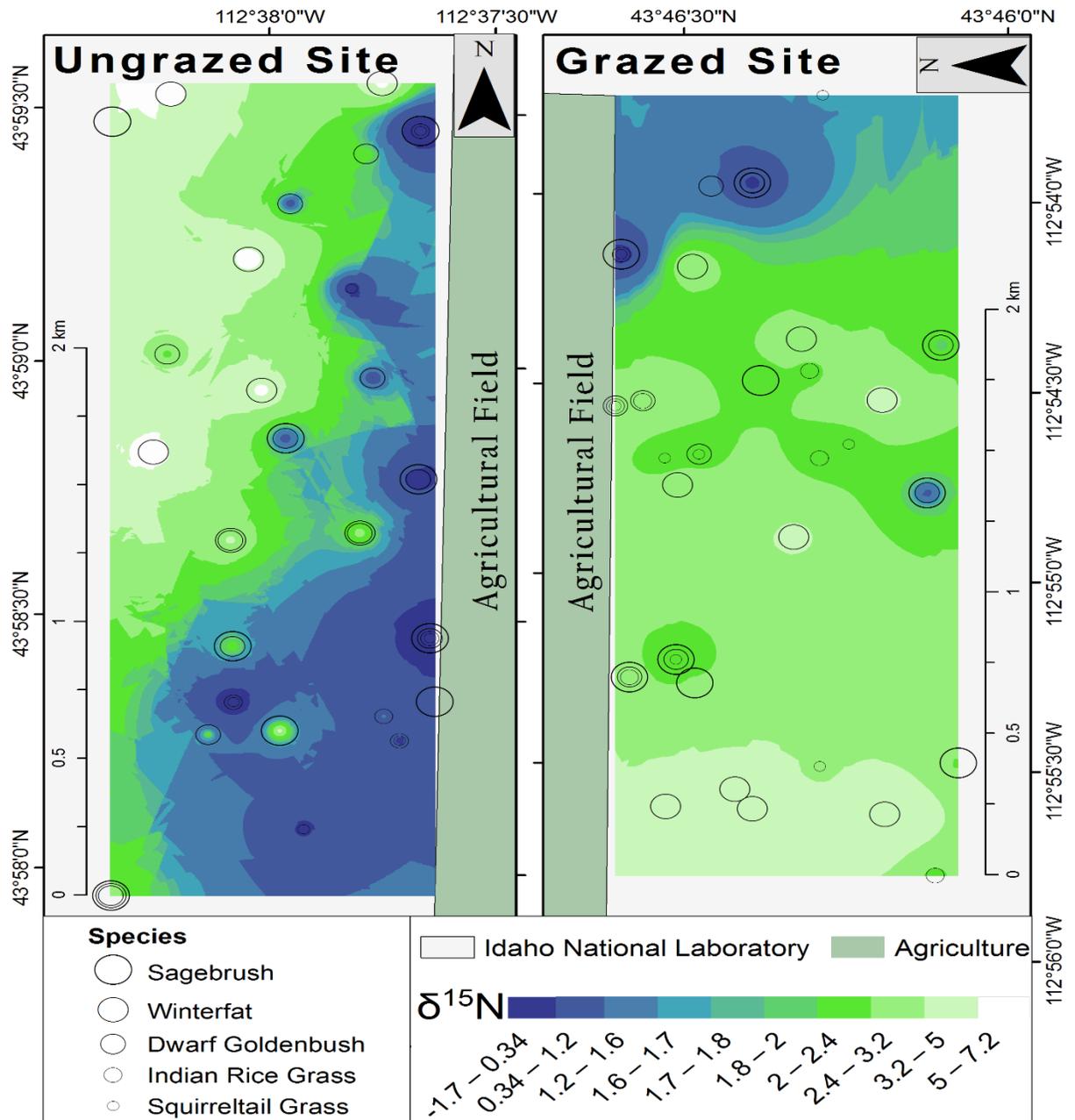


Figure 4. Inverse Distance Weighting (IDW) maps of  $\delta^{15}\text{N}$  on the Birch Creek (ungrazed) and Howe (grazed) based on samples obtained June-August, 2015, southeastern Idaho, USA. Map shows increasing levels of  $\delta^{15}\text{N}$  as distance increases from agriculture on the

ungrazed site, and increasing levels of  $\delta^{15}\text{N}$  moving from west to east on the grazed site. There are also agricultural fields within 300 m of the west end of the grazed site, and the grazing is heaviest on the east end, which is closest to the water tank.

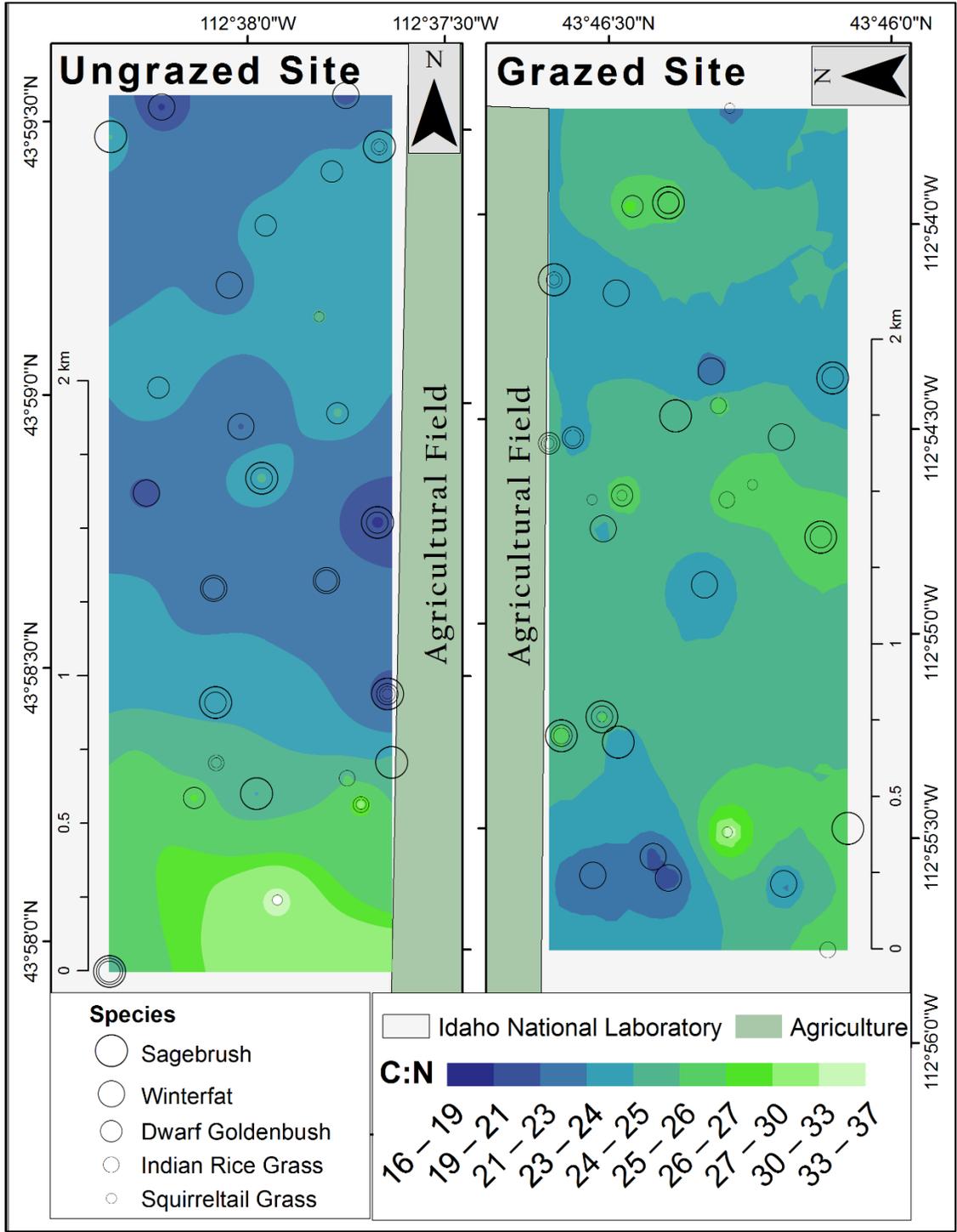


Figure 5. Inverse Distance Weighting (IDW) maps of C:N on the Birch Creek (ungrazed) and Howe (grazed) sites, southeastern Idaho, USA, summer 2015.

## CHAPTER 2

### EFFECTS OF ANTHROPOGENIC NITROGEN FLUXES ON SAGEBRUSH-STEPPE PLANT COMMUNITIES.

#### **Abstract**

We sampled vegetation cover, biomass, and diversity on two ostensibly similar sagebrush-steppe sites at the Idaho Nuclear Laboratory in east-central Idaho, USA, to elucidate effects of livestock grazing on cold desert plant communities, and possible effects of nutrient transfer from adjacent agricultural fields. We sampled 107 quadrats on two sites, one currently grazed and one that has not been grazed by livestock in ~70 years. We determined that plant cover, biomass, and diversity were all higher on the ungrazed site, although species richness was greater on the grazed site. We observed no evidence to support herbivore optimization. We also determined that there was no significant effect of distance from agriculture on cover and biomass of plants. Based on these results, and the results of an earlier study of nitrogen dynamics conducted at these same sites, we hypothesize that the differences in plant communities we observed between the two sites is the result of nitrogen export from the grazed site in the form of livestock biomass.

## **Introduction**

Ungrazed shrub-steppe is recognized as a critically endangered ecosystem that currently comprises ~2% of its pre-settlement extent (Noss et al., 1995). In the Intermountain West of the United States sagebrush-steppe formerly covered almost 100 million ha (Rowland et al., 2006), but has been reduced to ~45 million ha since European settlement. The remaining regions of sagebrush-steppe have been subjected to varying degrees of anthropogenic degradation. West (2000) characterized the current state of the ecosystem using a model with eight levels of retrogression, based on changes in vegetation from a pristine state. The pristine state (State I), only may exist large protected areas like Yellowstone National Park, if it exists at all. There are some areas in relictual condition (State II), which have escaped direct human influences, because there is limited access to water for livestock, or where grazing has been prohibited, often on federal military or nuclear reserves. Those sites differ from pristine conditions in that they lack the full suite of native animals and are subject to air pollution, and invasion by exotics. Much of the shrub-steppe is grazed at unsustainable levels (Fleischner 1994; Anderson and Inouye 2001), which may lead to states III-VIII. In State III (Stagnant Sagebrush), shrub species have come to dominate because of domestic livestock preferentially grazing on grasses and forbs. High stocking rates for livestock and range managers create State IV (Herb-dominated Stands) from stagnant sagebrush by controlled burns, chaining, and use of herbicides. State IV may be short-lived, and can return to relictual condition or deteriorate further depending on grazing regimes and fire occurrence. Stagnant Sagebrush also can deteriorate further into State V, (Desertified Sagebrush-Steppe), which is

characterized by taller, thicker woody shrubs, and an understory of introduced annual grasses. Introduced annual grasses increase fire frequency and cause the destruction of shrub cover (Dobkin and Sauder, 2004; Noss et al., 1995). Land management agencies have turned to planting introduced bunchgrasses such as crested wheatgrass (*Agropyron cristatum*) to reduce fire frequency and provide livestock forage, creating State VI (Introduced Bunch Grasslands). Native plant and animal communities, however, are badly damaged by this treatment (Lesica and DeLuca, 1996; Reynolds and Trost, 1980). Shrubs eventually will re-invade, resulting in Shrub Re-invaded Grasslands (State VII), a depauperate shrub and introduced grass community. When shrub-steppe burns, the usual result is State VIII (Annual Grassland) characterized by cheatgrass (*Bromus tectorum*) and Medusahead grass (*Taeniatherum caput-medusae*). West (2000) estimated that, as of 1999, 25% of shrub-steppe has been converted to exotic annual grassland, and efforts to restore introduced annual grassland to States II or III have been unsuccessful.

In addition to livestock, invasion of exotic annual grasses (West, 2000), fire (D'Antonio and Vitousek, 1992), agriculture (Brandt and Rickard, 1994), and development for oil and gas extraction (Avirmed et al., 2014) have damaged large areas of sagebrush-steppe.

Those activities may explain why, even when stocking rates of domestic grazing animals have been reduced, many areas have not returned to a relict state. Those areas may now represent new equilibria characterized by reduced populations of forbs and deep-rooted shrubs (Anderson and Inouye, 2001; West et al., 1984), or deteriorate further into exotic annual grasslands (Anderson and Inouye, 2001; West, 2000). Disrupted nitrogen cycles

may be a factor in the inability of those areas to return to their original state (Evans and Ehleringer, 1993).

Threats to the shrub-steppe ecosystem underscore the need to understand effects of anthropogenic influences on plant community composition, biomass and productivity in the sagebrush-steppe environment. The focus of our study was to examine effects of neighboring agricultural fields and livestock grazing on the composition and diversity of sagebrush-steppe plant communities. We hypothesized that differences in plant cover, biomass, diversity, and abundance of invasive species would manifest between our grazed and ungrazed sites.

Herbivore optimization (McNaughton, 1979) is a hypothesis that contends that low to moderate levels of herbivory increases plant productivity. This hypothesis has been well supported by other studies (Stewart et al., 2006) in ecosystems other than sagebrush-steppe, and has been used to justify high levels of grazing on public lands (Painter and Belsky, 1993). Stewart (2006) documented herbivore optimization in a montane ecosystem, but cautioned that herbivore optimization only takes place when there are low levels of herbivory and sufficient moisture and nutrients. Further, Painter and Belsky (1993) maintain that herbivore optimization only occurs under ideal conditions. We predict that, because of previously discussed effects of grazing, that vegetation cover and biomass will be greater on the ungrazed site compared to the grazed site. Thus, we predict that herbivore optimization is unlikely on our study sites. Relatedly, large herbivores have been known to act as keystone species, increasing plant diversity by

consuming species that would otherwise dominate plant communities (Bond, 1994; Knapp et al., 1999). We predict that species richness and diversity will be greater on the grazed site.

Along with grazing effects, we hypothesized that we would detect differences within sites in plant cover, biomass, and diversity at varying distances from adjacent agricultural fields. Specifically, because of transferal of nitrogen from the agricultural fields into the shrub-steppe (Long, Chapter 1) we predict that plant cover and biomass would decrease with increasing distance from agricultural fields. We assume that our study sites are typical of relatively intact grazed and ungrazed (Stage II or III) sagebrush-steppe.

## **Methods**

### **Study Site**

The study area is located in southeastern Idaho, USA (Fig. 1) on the Idaho National Laboratory (INL). There were two sites; one located in the northeast corner of the INL (Birch Creek), and the other southeast of the town of Howe. Dimensions of study sites were approximately  $2 \times 3$  km (Fig. 1). We chose to sample such large study sites to ensure that our sampling was representative of the sagebrush-steppe ecosystem. Elevations ranged from 1461 to 1463 m on the grazed site, and 1466 to 1473 m on the ungrazed site. Vegetation on the study sites is cold desert shrub-steppe (Anderson et al., 1996; Anderson and Inouye, 2001) and topography at both sites is flat. Anderson (1996) provides a complete flora and vegetation description of the INL. Based on the characterization of vegetation state in the shrub-steppe ecosystem by West (2000), both

sites are in State III—Stagnant Sagebrush. The grazed site has a herbaceous understory dominated by needle and thread grass, but forbs have never recovered from drought and overgrazing in the 1930-40s (Anderson and Inouye, 2001) The grazed site currently is grazed at 13.8 AUM (personal communication, J. Smith, Bureau of Land Management, 2016). An AUM (Animal Unit Month) is the amount of forage needed by a cow-calf pair for 1 month. This value usually is calculated at 12 kg of forage per day. The ungrazed site near has not been grazed since the INL was established in 1947 (Personal communication, J.Shive, Western Advantage, 2015). Grazing by native herbivores was sparse on both sites. Those herbivores included North American elk (*Cervus elaphus*), mule deer (*Odocoileus hemionus*), pronghorn (*Antilocapra americana*), black-tailed jackrabbits (*Lepus californicus*), greater sage grouse (*Centrocercus urophasianus*), pygmy rabbits (*Brachylagus idahoensis*), and other smaller herbivores. Annual precipitation at the grazed site averaged 15.7 cm from 2007 to 2015 (NOAA). Rainfall at Blue Dome, the nearest weather station to the ungrazed site, averaged 16.4 cm from 2007 to 2015 (personal communication, Jason Rich NOAA, 2016). Data for snowfall from that station is not available, but snowfall at the INL Central Facility averaged 65 cm annually. The highest average rainfall for the region is during the months of May and June, with an average during those months of 3 cm. The lowest average precipitation is during July with an average of 1 cm. Highest average snowfall is during December, which averages 16 cm. The hottest month is July, with an average maximum of 28° C, and an average minimum of 9.5° C. The coldest month is December, with an average minimum of -33° C, and an average maximum of 8° C. The predominant soil type on the grazed site is Sparmo silt loam, which is a well-drained soil with an A horizon averaging 23 cm deep.

The predominant soil type on the ungrazed site is Whiteknob gravelly loam, which is an excessively well drained soil, and has an A horizon averaging 12.5 cm deep. When irrigated, both soil types are considered to provide prime farmland. (<http://www.casoilresource.lawr.ucdavis.edu/gma>). Frost-free days on the INL average 115 days per annum. (<http://www.cals.uidaho.edu/edcomm/pdf/bul/bul0857.pdf>). The growing season occurs mid-April to late September. Both sites are bordered by agriculture. The grazed site is bordered on the north by both wheat and alfalfa fields, and on the west side of the site there are alfalfa fields. There is no permanent surface water on the INL, so water is trucked in by ranchers to a point approximately 2 km SE of the SE corner of that study site. The ungrazed site is bordered on the east by wheat and alfalfa fields, and ranchers are not allowed to transport water into this area. Alfalfa, a nitrogen-fixing legume, is fertilized only with phosphorus (P). Wheat is fertilized with both nitrogen (N) and phosphorus.

### **Sampling Methods**

Sampling at the two sites was conducted during summer 2015. The sites were divided into 10 intervals, at increasing 100-m distances from an agricultural border with an 11<sup>th</sup> interval placed 2 km from the agricultural field. Five 5 x 5-m quadrats were placed randomly within each 100-m interval, for a total 55 sampling quadrats within each study site. Three transect lines were placed across each quadrat spaced 125 cm apart. Direction (N-S or E-W) was decided by a coin flip. Cover for each plant was measured from start to end of where the plant intersected a transect line. If there was a gap of >5 cm in an

individual plant along the transect, the distance across the gap was omitted from the calculation of cover. For plants other than shrubs, plants that touched a transect line were harvested by clipping from ground level upward. For each shrub that was transected with the vertical projection of a transect line, 25 leaders of current annual growth, including leaves and stems, were clipped. Plant samples were dried at 50° C until weight remained constant for 24 h. Samples were weighed to the nearest 0.1 g on a Fisher Scientific Industries SLF501-US scale (Pittsburgh, Pennsylvania, USA). Given the presumed similarity of the two sites to the general cold steppe landscape, and the similarity of sites to each other, save for grazing, we assumed for analyses that quadrats were replicates of distance-grazing treatments, with inferable extensions to the region.

### **Data Analyses**

We tested for differences in percent cover and of biomass between study sites and among distance categories using two-way ANOVA with site and distance from agricultural fields as main effects, and plant species as independent variables. All statistical analyses were performed in R version 3.01 (R Core Development Team, Vienna). The `indval()` from R-package `labdsv` (Roberts 2016) function, which applies indicator-species analysis (Dufrene and Legendre 1997) was used to inferentially examine differences in abundance of plant species between the two sites and across sampling intervals. Evenness was calculated manually (Buzas and Gibson, 1969) We used  $\alpha = 0.05$  as the significance level; all statistical tests were two tailed. We conducted Non Metric Multidimensional Scaling on the correlation matrix to evaluate the composite effects of site, distance, and type of plant (Kruskal and Wish 1978, (Aho, 2013; Gardener, 2014). Principal

Components Analysis (Hotelling, 1933) was used to measure relative importance of NMDS axes. Fligner-Killeen tests (Fligner and Killeen, 1976) were used to verify assumptions of homogeneity of variance. For those plant types that did not meet assumptions for homoscedasticity, we used Brunner-Dette-Munk (Brunner et al., 1997) tests in place of ANOVA to test differences in cover and biomass.

Inverse Distance Weighting (IDW) was used to provide bivariate representations of the variation in vegetation cover and biomass at two sites and across distances from agricultural lands. IDW interpolates heteroscedastic data without the need for transformation, while maintaining spatial interpolation accuracy (Isaaks et al. 1989, Cooke et al., 1993; Weber and Englund, 1992). IDW was more appropriate than the stochastic method of kriging, because kriging requires assumptions of stationarity and isotropy (Cressie, 1985). Additionally, an insufficient sample size was available to detrend data for universal kriging, which assumes a trend in data (Peterson et al., 2013; Li and Heap 2014). We used Moran's I (Bolstad 2012, Sawada 2015) to identify the spatial character of data (e.g., clustered, random, or dispersed) at different spatial scales. We used a High-Low Clustering Getis-Ord General G tool to examine specific clustering characteristics. All IDW analyses were performed within ArcMap 10.3.3 (ESRI, Redlands, California, USA).

## Results

Our aim was to quantify differences in plant communities between grazed and ungrazed sites, and across distance from agricultural fields. Plant cover, biomass, and diversity (Appendix 1) are standard metrics used to describe plant communities. The correlation between percent cover and biomass of plant species within plots was very strong ( $r_s = 0.865$ ,  $p < 0.00001$ ). Hence, we only report results here for percent cover. Percent cover was significantly greater for all plants combined on the ungrazed compared with the grazed site ( $F_{1,85} = 18.95$ ,  $p < 0.00004$ ), but there was no significant effect of distance from agriculture ( $F_{10,85} = 1.45$ ,  $p = 0.18$ ). Bryophytes exhibited significantly greater cover on the ungrazed compared with the grazed site ( $F_{1,40,41} = 55.35$ ,  $p < 4.34e-09$ ); a significant negative relationship with distance from agriculture ( $F_{7,40,41} = 2.31$ ,  $p < 0.05$ ) also occurred. The percent cover of forbs was slightly higher on the grazed site compared with the ungrazed site but sites did not differ significantly ( $F_{1,85} = 2.37$ ,  $p = 0.13$ ). There was no effect of distance from agriculture on cover of forbs ( $F_{10,85} = 1.72$ ,  $p = 0.09$ ). For grasses, percent cover was significantly greater on the ungrazed site than on the grazed site ( $F_{1,85} = 18.21$ ,  $p < 0.00006$ ), but there was no significant effect for distance from agriculture ( $F_{10,85} = 0.51$ ,  $p < 0.88$ ). Cover of macrobiotic crust was significantly greater on the ungrazed site ( $F_{1,30,4} = 10.43$ ,  $p < 0.003$ ), but exhibited no significant effect of distance from agriculture ( $F_{6,5,36,3} = 1.62$ ,  $p = 0.17$ ). Significantly greater cover of succulents (cactus) occurred on the ungrazed site ( $F_{1,22,5} = 11.56$ ,  $p < 0.003$ ), but again, no significant effect of distance occurred ( $F_{5,4,22,5} = 1.62$ ,  $p = 0.17$ ). Cover of shrubs did not

differ significantly between sites ( $F_{10,85} = 0.61$ ,  $p < 0.44$ ) or among distance ( $F_{10,85} = 0.78$ ,  $p < 0.66$ )

Species richness was greater on the grazed than the ungrazed site ( $F_{1,85} = 4.34$ ,  $p < 0.041$ ). We collected 32 species of plants on the grazed site and 27 species on the ungrazed site. Nonetheless, species diversity (Shannon's  $e^H$ ) (Hill, 1973) was greater on the ungrazed site (3.429) and lower (2.947) on the grazed site ( $F_{1,85} = 4.43$ ,  $p < 0.04$ ). Evenness on the ungrazed site was 0.13, and 0.09 on the grazed site. The ungrazed site was characterized by significantly greater cover of bryophyte (*Tortula ruralis*), cactus (*Opuntia polyacantha*), dwarf goldenbush (*Ericameria nana*), needle-and-thread grass (*Hesperostipa comata*), macrobiotic crust, big sagebrush (*Artemisia tridentata*), and Hood's phlox (*Phlox hoodia*). The grazed site was characterized by significantly greater abundance of Indian ricegrass (*Achnatherium hymenoides*), (*Atriplex falcata*), slimleaf goosefoot (*Chenopodium leptophyllum*), western wheatgrass (*Pascopyrum smithii*), gray rabbitbrush (*Ericameria nausiosa*), and squirreltail grass (*Elymus elymoides*) (Appendix 1).

The Non-metric Multi Dimensional Scaling analysis (Figure 2) performed best with three axes, with all stressors scoring between 0.16 and 0.17. Data points for the two sites are strongly separated across all three axes. Principal Components Analysis of the NMDS scores indicates that the NMDS explained 90% of the variation in our samples. Values for the ungrazed site are heavily clustered negatively on Axis 1, indicating significant differences between the two sites. Forbs and shrubs were mostly neutral on Axis 1,

however. All forage classes except forbs exhibited slightly negative values on Axis 2, whereas forbs showed a stronger positive value. Forbs and grasses also showed strongly positive values on Axis 3, in contrast with the other forage classes.

The Inverse Distance Weighting maps served to illustrate the differences between sites. The maps also elucidate the lack of significant differences across intervals, because the intervals run parallel to the agricultural border, and there is no corresponding pattern to the plant cover. The IDW maps also illustrate the patchy nature of the vegetation cover, especially on the grazed site.

## **Discussion**

Our results strongly support our predictions that vegetation cover and biomass would be greater on the ungrazed site than the grazed site. No obvious evidence for herbivore optimization (*sensu* McNaughton, 1979; Stewart et al., 2006) was observed on the grazed site, even though the stocking rate is lower than what is typical for grazing allocations in sagebrush-steppe (personal communication, J. Smith, Bureau of Land Management).

Cover of grasses was significantly greater on the ungrazed site than the grazed site.

Possible explanations for those outcomes include that stocking rates are too high for herbivore optimization, or that the study site is too arid to support that process (Stewart et al., 2006). The IDW maps illustrate differences in vegetation and nitrogen dynamics between the two sites, and depict the patchy nature of the sagebrush-steppe vegetation.

The IDW map also illustrates the effect of the location of the watering tank on the vegetation in the grazed site. The water tank is located 2 km south of the southeast corner

of the grazed area. The vegetation cover increases from the southeast corner to the northeast corner.

Our data did not support our prediction that vegetation cover would decrease with distance from the agricultural fields. This contrasts strongly with our results from our stable isotope study (Long, Chapter 1), wherein we documented that the percentage of nitrogen in plant tissues decreased with distance from agriculture. We hypothesized that C:N would be an indicator of nitrogen availability, and with decreasing C:N we would observe increases in plant cover and biomass. There appears to be a closer inverse relationship between  $\delta^{15}\text{N}$  and vegetation cover and biomass than with C:N. Plant cover was much less (~73%) and  $\delta^{15}\text{N}$  (Long, Chapter 1) significantly greater on the grazed site, indicating that N limitation is a significant factor in the variation between the grazed and ungrazed sites. Increased levels of  $\delta^{15}\text{N}$  in plant tissues has been shown to be an indicator of low nitrogen availability (Dawson et al., 2002; Peterson and Fry, 1987).

A possible cause of the differences we detected is export of nitrogen from the shrub-steppe ecosystem in the form of livestock biomass (Long, Chapter 1). For every 100 kg of mass gained by cattle grazing on the sagebrush-steppe, nitrogen comprises approximately 3 kg (Parmenter and MacMahon, 2009). When livestock are shipped to market, a net export of nitrogen occurs, consisting of approximately 3% of the total mass of livestock removed from the ecosystem, and that nitrogen is not replaced, even though some supplemental feeding of livestock may occur.

Earlier we reported that the most significant result for distance from agriculture was the effect on C:N (Long, Chapter 1). C:N increased with distance from agriculture. That effect was most likely caused by both large and small herbivores feeding in the agricultural fields and transporting nitrogen to the adjacent shrub-steppe in the form of urine, feces, and carcasses. In this process, animals improve the habitat in the neighboring shrub-steppe for small herbivores by improving the quality of the forage. This effect decreases with distance from agriculture, which makes the area near the agricultural fields even more attractive to herbivores in relation to shrub-steppe that has no neighboring agriculture.

Contrary to our prediction species richness was greater on the grazed than ungrazed site. Shannon-Weiner diversity, however, was greater on the ungrazed site because of greater evenness. This result may stem from a keystone effect caused by cattle on diversity of plants.

Evans and Ehleringer, (1993) demonstrated that cryptobiotic crusts were the primary nitrogen sources in shrub-steppe on the Colorado Plateau. Disturbance of those crusts caused a net loss of nitrogen from the ecosystem and elevated levels of  $\delta^{15}\text{N}$ . We observed a significant difference in cover of cryptobiotic crust between our two sites, with crusts being almost entirely absent from the grazed site. Other plants that were relatively abundant on the ungrazed site that were rare on the grazed site were bryophytes

and succulents. The bryophytes were by far the most nitrogen rich plants in the study (Long, Chapter 1). The absence of bryophytes indicates that cattle are either consuming cactus and bryophyte, or that the grazed site lacks the active nitrogen sufficient to support cactus and bryophytes.

Many species of small herbivores have declined from historic levels in sagebrush steppe ecosystems including sage grouse, pygmy rabbits and jackrabbits. Although sage grouse are not currently federally listed, populations of greater sage grouse are imperiled (Finch et al., 2016; Knick et al., 2003). Pygmy rabbits are currently federally listed in the state of Washington, and were considered for a national listing in 2010. Those lagomorphs are known to avoid areas where cattle graze because cattle reduce grass cover and reduce the nutritional quality of what they leave behind (Siegel Thines et al., 2004). Jackrabbits have failed to rebound to historic levels since the last irruption in 1981 (Anderson and Inouye, 2001). Jackrabbits normally increase and decrease on a 10-year cycle. Peaks in population typical of this cycle have not been evident for 35 years. Small herbivores have less efficient metabolisms (Kleiber, 1961), burning more calories per gram of mass, and require high quality forage—high-quality forage is defined as forage with high nitrogen content and lower carbon content (Demment and Van Soest, 1985). Carbon in plant tissues is mostly fiber, which require gut symbionts to efficiently digest (Soest, 1982). Because cattle have large rumens and can subsist on low-quality forage, and the only control on the grazing pressure is the stocking rate; cattle can consume most of the high-quality forage, and then continue to persist on the low-quality plants. Small herbivores,

on the other hand, require high-quality forage, and have few alternatives when such resources are no longer available.

There is greater cover and biomass on the ungrazed site, greater abundance of nitrogen-rich plants such as bryophytes, and a slight but detectable increase in average nitrogen content on the ungrazed site (Long, Chapter 1). Thus, we hypothesize that the ungrazed site has a greater overall nitrogen budget than the grazed site. This result indicates the ungrazed site provides superior habitat for small herbivores, with greater cover for protection from predators, and higher quality forage, especially if bryophytes are included in their diets.

Another possible cause of the differences we detected is export of nitrogen from the shrub-steppe ecosystem in the form of livestock biomass (Long, Chapter 1). For every 100 kg of mass gained by cattle grazing on the sagebrush-steppe, nitrogen comprises approximately 3 kg. When livestock are shipped to market, a net export of nitrogen occurs, consisting of approximately 3% of the total mass of livestock removed from the ecosystem, and that nitrogen is not replaced, even though some supplemental feeding of livestock may occur.

Our study strongly supports our hypothesis that plant cover and biomass are negatively affected by livestock grazing. Because our study sites are very similar in terms of climate, terrain, and edaphic factors, we believe that this effect is caused by effects of livestock

grazing on nitrogen dynamics, specifically by export of nitrogen from the ecosystem in the form of livestock biomass. Studies of shrub-steppe systems that are very close to pristine condition, with a full suite of herbivores and predators, such as the Lamar Valley in Yellowstone National Park would be helpful in further clarifying the nutrient export hypothesis proposed herein.

### **Acknowledgements**

Funding was provided by a grant from the College of Science and Engineering at Idaho State University. Mariah Blackhorse assisted with sampling and other field work. Dr. Sarah Godsey assisted with the IDW maps. Johanna Thalmann provided invaluable assistance in resolving Microsoft office problems. Sunshine Denney provided space for sample storage.

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**Appendix I.** List of species sampled in the study June to August, 2015, with summary statistics. Includes forage class, mean, standard deviation, minimum and maximum for percent cover and biomass for each site.

Species	Class	Origin	Birch Creek												Howe											
			Cover%						Biomass(gm/m <sup>2</sup> )						Cover%						Biomass(gm/m <sup>2</sup> )					
			$\bar{x}$	SD	Min	Max	$\bar{x}$	SD	Min	Max	$\bar{x}$	SD	Min	Max	$\bar{x}$	SD	Min	Max	$\bar{x}$	SD	Min	Max				
Achnatherum hymenoides	G	N	0.924	1.917	0.133	10.2	####	0.531	0	2.004	2.006	2.578	0.067	9.2	0.583	0.99	0.004	5.012								
Alyssum desertorum	F	I	0.007	0.054	0.4	0.4	####	0.002	0.01	0.012	0	0	0	0	0.000	0	0	0								
Artemisia arbuscula	S	N	0.022	0.085	0.133	0.467	####	0.1	0.03	0.68	0.015	0.062	0.2	0.333	0.009	0.048	0.032	0.324								
Artemisia frigida	S	N	0.011	0.081	0.6	0.6	####	0.005	0.04	0.04	0	0	0	0	0.000	0	0	0								
Artemisia tridentata	S	N	7.915	6.579	0.333	25.67	####	0.823	0.03	3.68	5.41	7.569	0.067	38.07	0.702	0.849	0.04	3.348								
Astragalus filipes	F	N	0	0	0	0	####	0	0	0	0.002	0.018	0.133	0.133	0.001	0.009	0.064	0.064								
Atriplex canescens	S	N	0	0	0	0	####	0	0	0	0.09	0.483	1.8	3.133	0.024	0.133	0.42	0.9								
Atriplex falcata	S	N	0.027	0.141	0.6	0.867	####	0.017	0	0.128	2.377	4.352	0.067	18.73	1.266	2.331	0.004	12.016								
Bromus tectorum	G	I	0.04	0.297	2.2	2.2	####	0.091	0.67	0.672	0.004	0.027	0.2	0.2	0.000	0.003	0.02	0.02								
Syntrichia ruralis	B	N	2.247	2.855	0.267	11.13	####	5.883	0.05	22.64	0.184	0.821	0.333	5.467	0.064	0.233	0.108	1.116								
Chenopodium leptophyllum	F	N	0	0	0	0	####	0	0	0	0.063	0.17	0.067	0.8	0.008	0.029	0.004	0.172								
Chrysothamnus viscidiflorus	S	N	0.067	0.494	3.667	3.667	####	0.033	0.24	0.244	0.008	0.063	0.467	0.467	0.001	0.005	0.036	0.036								
Descurainia pinnata	F	N	0.001	0.009	0.067	0.067	####	5E-04	0	0.004	0.005	0.036	0.267	0.267	0.001	0.004	0.028	0.028								
Elymus elymoides	G	N	0.177	0.382	0.267	2.333	####	0.16	0	0.98	0.762	1.449	0.067	5.933	0.117	0.214	0.012	0.936								
Elymus lanceolatus	G	N	0	0	0	0	####	0	0	0	0.113	0.438	0.067	2.467	0.016	0.076	0.02	0.52								
Elymus smithii	G	N	0	0	0	0	####	0	0	0	0.105	0.782	5.8	5.8	0.027	0.2	1.484	1.484								
Eriogonum microthecum	S	N	0.002	0.018	0.133	0.133	####	0.006	0.04	0.044	0.018	0.135	1	1	0.007	0.054	0.4	0.4								
Ericameria nauseosa	S	N	0	0	0	0	####	0	0	0	0.45	1.516	0.333	9.067	0.094	0.285	0.096	1.5								
Ericameria nana	S	N	3.727	5.961	0.067	29.87	####	0.645	0	3.876	0.858	2.187	0.067	13	0.111	0.227	0.028	1.24								
Eriogonum ovalifolium	F	N	0.01	0.072	0.533	0.533	####	0.298	2.21	2.212	0.01	0.072	0.533	0.533	0.001	0.005	0.04	0.04								
Erigeron pumilus	F	N	0.002	0.018	0.133	0.133	####	0.006	0.04	0.044	0	0	0	0	0.000	0	0	0								
Festuca idahoensis	G	N	0	0	0	0	####	0	0	0	0.028	0.207	1.533	1.533	0.004	0.027	0.2	0.2								
Gutierrezia sarothrae	S	N	0.046	0.299	0.333	2.2	####	0.174	0.15	1.284	0.038	0.212	0.2	1.533	0.011	0.058	0.084	0.404								
Halogeton glomeratus	F	N	1.956	4.519	0.067	22.73	####	1.758	0	10.1	3.943	7.541	0.067	32.53	0.833	1.58	0.004	7.16								
Hesperostipa comata	G	N	8.092	9.703	0.2	41.53	####	2.071	0	8.604	0.15	0.45	0.067	2.2	0.026	0.074	0.004	0.308								
Krascheninnikovia lanata	S	N	1.996	3.638	0.067	12.07	####	0.826	0.16	3.272	3.502	6.476	0.067	24.47	1.074	2.251	0.036	9.264								
Linanthus pungens	S	N	0.002	0.018	0.133	0.133	####	0.029	0.21	0.212	0	0	0	0	0.000	0	0	0								
MBC	L	N	0.212	0.667	0.333	4.333	####	0	0	0	0.019	0.104	0.4	0.667	0.0	0.0	0.0	0.0								
Opuntia polyacantha	S	N	0.344	0.836	0.067	3.533	####	3.987	1.75	15.55	0.045	0.233	1.2	1.267	0.237	1.459	2.428	10.588								
Pascopyrum smithii	G	N	0	0	0	0	####	0	0	0	0.256	0.859	0.133	4.467	0.044	0.128	0.044	0.728								
Phlox hoodii	F	N	0.128	0.485	0.333	2.467	####	0.167	0	1.208	0	0	0	0	0.000	0	0	0								
Phlox longifolia	F	N	0	0	0	0	####	0	0	0	0.01	0.072	0.533	0.533	0.000	5E-04	0.004	0.004								
Poa secunda	G	N	0.015	0.08	0.267	0.533	####	0.072	0.19	0.5	0	0	0	0	0.000	0	0	0								
Salsolakali	F	I	0.469	2.642	0.067	18.67	####	0.671	0	4.472	0.015	0.091	0.133	0.667	0.002	0.016	0.008	0.12								
Schoenocrambe linifolia	F	N	0.017	0.078	0.133	0.467	####	0.003	0.01	0.016	0.027	0.156	0.067	1.133	0.001	0.006	0.004	0.032								
Sisymbrium altissimum	F	I	0	0	0	0	####	0	0	0	0.155	1.027	0.333	7.6	0.019	0.134	0.016	0.992								
Sphaeralcea munroana	F	N	0.032	0.122	0.133	0.733	####	0.039	0	0.276	0.07	0.275	0.067	1.867	0.011	0.05	0.008	0.356								
Tragopogon dubius	F	N	0	0	0	0	####	0	0	0	0.002	0.018	0.133	0.133	0.001	0.007	0.052	0.052								

Species	Class	Origin	Birch Creek								Howe							
			Cover%				Biomass(gm/m <sup>2</sup> )				Cover%				Biomass(gm/m <sup>2</sup> )			
			$\bar{X}$	SD	Min	Max	$\bar{X}$	SD	Min	Max	$\bar{X}$	SD	Min	Max	$\bar{X}$	SD	Min	Max
<i>Achnatherum hymenoides</i>	G	N	0.924	1.917	0.133	10.2	0.2959	0.531	0	2.004	2.006	2.578	0.067	9.2	0.583	0.99	0.004	5.012
<i>Alyssum desertorum</i>	F	I	0.007	0.054	0.4	0.4	0.0002	0.002	0.01	0.012	0	0	0	0	0.000	0	0	0
<i>Artemisia arbuscula</i>	S	N	0.022	0.085	0.133	0.467	0.0207	0.1	0.03	0.68	0.015	0.062	0.2	0.333	0.009	0.048	0.032	0.324
<i>Artemisia frigida</i>	S	N	0.011	0.081	0.6	0.6	0.0007	0.005	0.04	0.04	0	0	0	0	0.000	0	0	0
<i>Artemisia tridentata</i>	S	N	7.915	6.579	0.333	25.67	0.8545	0.823	0.03	3.68	5.41	7.569	0.067	38.07	0.702	0.849	0.04	3.348
<i>Astragalus filipes</i>	F	N	0	0	0	0	0.0000	0	0	0	0.002	0.018	0.133	0.133	0.001	0.009	0.064	0.064
<i>Atriplex canescens</i>	S	N	0	0	0	0	0.0000	0	0	0	0.09	0.483	1.8	3.133	0.024	0.133	0.42	0.9
<i>Atriplex falcata</i>	S	N	0.027	0.141	0.6	0.867	0.0024	0.017	0	0.128	2.377	4.352	0.067	18.73	1.266	2.331	0.004	12.016
<i>Bromus tectorum</i>	G	I	0.04	0.297	2.2	2.2	0.0122	0.091	0.67	0.672	0.004	0.027	0.2	0.2	0.000	0.003	0.02	0.02
<i>Syntrichia ruralis</i>	B	N	2.247	2.855	0.267	11.13	3.4648	5.883	0.05	22.64	0.184	0.821	0.333	5.467	0.064	0.233	0.108	1.116
<i>Chenopodium leptophyllum</i>	F	N	0	0	0	0	0.0000	0	0	0	0.063	0.17	0.067	0.8	0.008	0.029	0.004	0.172
<i>Chrysothamnus viscidiflorus</i>	S	N	0.067	0.494	3.667	3.667	0.0044	0.033	0.24	0.244	0.008	0.063	0.467	0.467	0.001	0.005	0.036	0.036
<i>Descurainia pinnata</i>	F	N	0.001	0.009	0.067	0.067	0.0001	5E-04	0	0.004	0.005	0.036	0.267	0.267	0.001	0.004	0.028	0.028
<i>Elymus elymoides</i>	G	N	0.177	0.382	0.267	2.333	0.0435	0.16	0	0.98	0.762	1.449	0.067	5.933	0.117	0.214	0.012	0.936
<i>Elymus lanceolatus</i>	G	N	0	0	0	0	0.0000	0	0	0	0.113	0.438	0.067	2.467	0.016	0.076	0.02	0.52
<i>Elymus smithii</i>	G	N	0	0	0	0	0.0000	0	0	0	0.105	0.782	5.8	5.8	0.027	0.2	1.484	1.484
<i>Eriogonum microthecum</i>	S	N	0.002	0.018	0.133	0.133	0.0008	0.006	0.04	0.044	0.018	0.135	1	1	0.007	0.054	0.4	0.4
<i>Ericameria nauseosa</i>	S	N	0	0	0	0	0.0000	0	0	0	0.45	1.516	0.333	9.067	0.094	0.285	0.096	1.5
<i>Ericameria nana</i>	S	N	3.727	5.961	0.067	29.87	0.4048	0.645	0	3.876	0.858	2.187	0.067	13	0.111	0.227	0.028	1.24
<i>Eriogonum ovalifolium</i>	F	N	0.01	0.072	0.533	0.533	0.0402	0.298	2.21	2.212	0.01	0.072	0.533	0.533	0.001	0.005	0.04	0.04
<i>Erigeron pumilus</i>	F	N	0.002	0.018	0.133	0.133	0.0008	0.006	0.04	0.044	0	0	0	0	0.000	0	0	0
<i>Festuca idahoensis</i>	G	N	0	0	0	0	0.0000	0	0	0	0.028	0.207	1.533	1.533	0.004	0.027	0.2	0.2
<i>Gutierrezia sarothrae</i>	S	N	0.046	0.299	0.333	2.2	0.0260	0.174	0.15	1.284	0.038	0.212	0.2	1.533	0.011	0.058	0.084	0.404
<i>Halogeton glomeratus</i>	F	N	1.956	4.519	0.067	22.73	0.6623	1.758	0	10.1	3.943	7.541	0.067	32.53	0.833	1.58	0.004	7.16
<i>Hesperostipa comata</i>	G	N	8.092	9.703	0.2	41.53	1.7328	2.071	0	8.604	0.15	0.45	0.067	2.2	0.026	0.074	0.004	0.308
<i>Krascheninnikovia lanata</i>	S	N	1.996	3.638	0.067	12.07	0.4721	0.826	0.16	3.272	3.502	6.476	0.067	24.47	1.074	2.251	0.036	9.264
<i>Linanthus pungens</i>	S	N	0.002	0.018	0.133	0.133	0.0039	0.029	0.21	0.212	0	0	0	0	0.000	0	0	0
MBC	L	N	0.212	0.667	0.333	4.333	0.0000	0	0	0	0.019	0.104	0.4	0.667	0.0	0.0	0.0	0.0
<i>Opuntia polyacantha</i>	S	N	0.344	0.836	0.067	3.533	1.8755	3.987	1.75	15.55	0.045	0.233	1.2	1.267	0.237	1.459	2.428	10.588
<i>Pascopyrum smithii</i>	G	N	0	0	0	0	0.0000	0	0	0	0.256	0.859	0.133	4.467	0.044	0.128	0.044	0.728
<i>Phlox hoodii</i>	F	N	0.128	0.485	0.333	2.467	0.0321	0.167	0	1.208	0	0	0	0	0.000	0	0	0
<i>Phlox longifolia</i>	F	N	0	0	0	0	0.0000	0	0	0	0.01	0.072	0.533	0.533	0.000	5E-04	0.004	0.004
<i>Poa secunda</i>	G	N	0.015	0.08	0.267	0.533	0.0126	0.072	0.19	0.5	0	0	0	0	0	0	0	0
<i>Salsola kali</i>	F	I	0.469	2.642	0.067	18.67	0.1364	0.671	0	4.472	0.015	0.091	0.133	0.667	0.002	0.016	0.008	0.12
<i>Schoenocrambe linifolia</i>	F	N	0.017	0.078	0.133	0.467	0.0007	0.003	0.01	0.016	0.027	0.156	0.067	1.133	0.001	0.006	0.004	0.032
<i>Sisymbrium altissimum</i>	F	I	0	0	0	0	0.0000	0	0	0	0.155	1.027	0.333	7.6	0.019	0.134	0.016	0.992
<i>Sphaeralcea munroana</i>	F	N	0.032	0.122	0.133	0.733	0.0071	0.039	0	0.276	0.07	0.275	0.067	1.867	0.011	0.05	0.008	0.356
<i>Tragopogon dubius</i>	F	N	0	0	0	0	0.0000	0	0	0	0.002	0.018	0.133	0.133	0.001	0.007	0.052	0.052

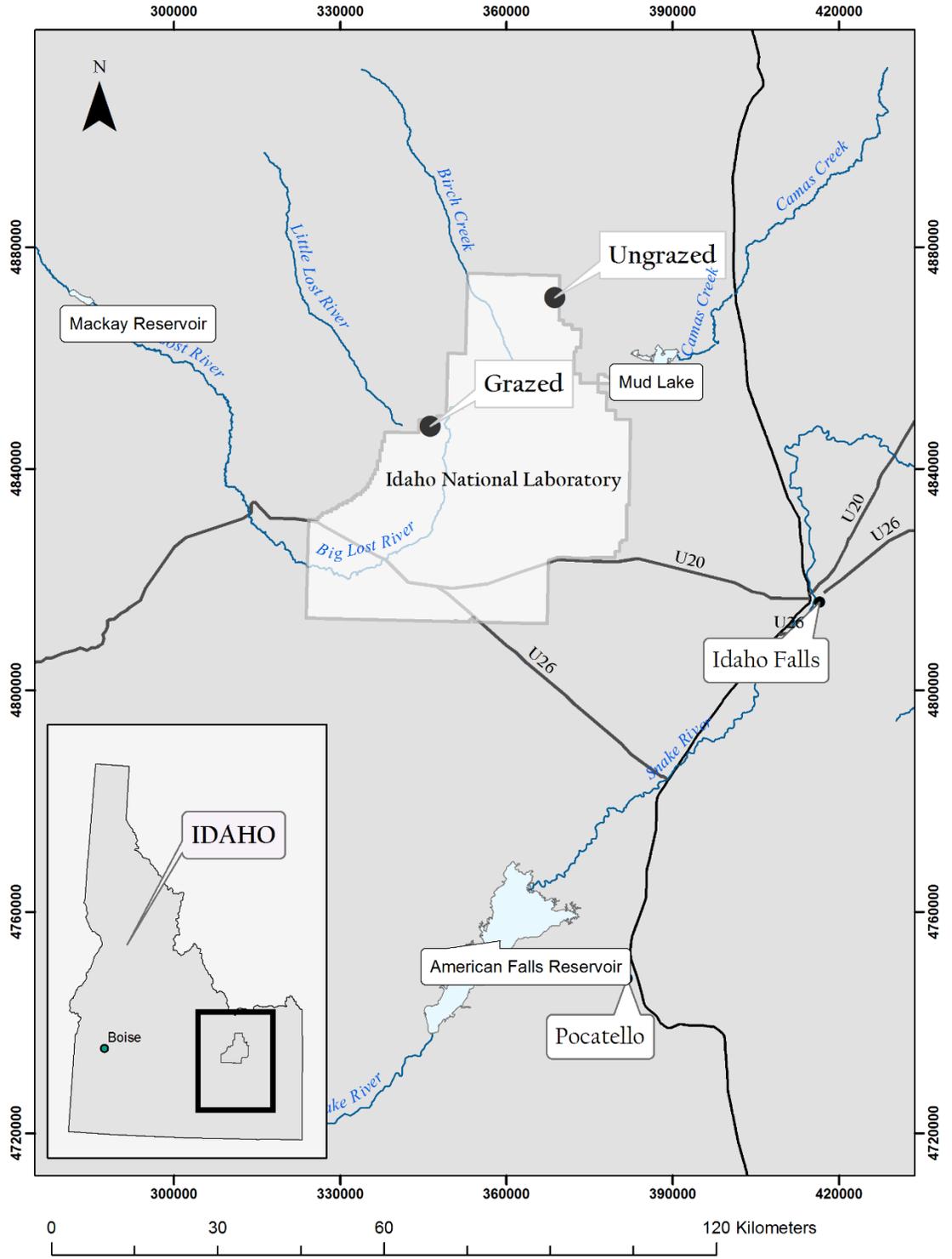


Figure 1. Extant map of study-site locations with inset of Idaho, USA. The Big Lost River and Birch Creek no longer flow within the boundaries of the INL, because of irrigation withdrawals. As a result, for most of the year, there is no natural surface water on the Idaho National Laboratory (INL).

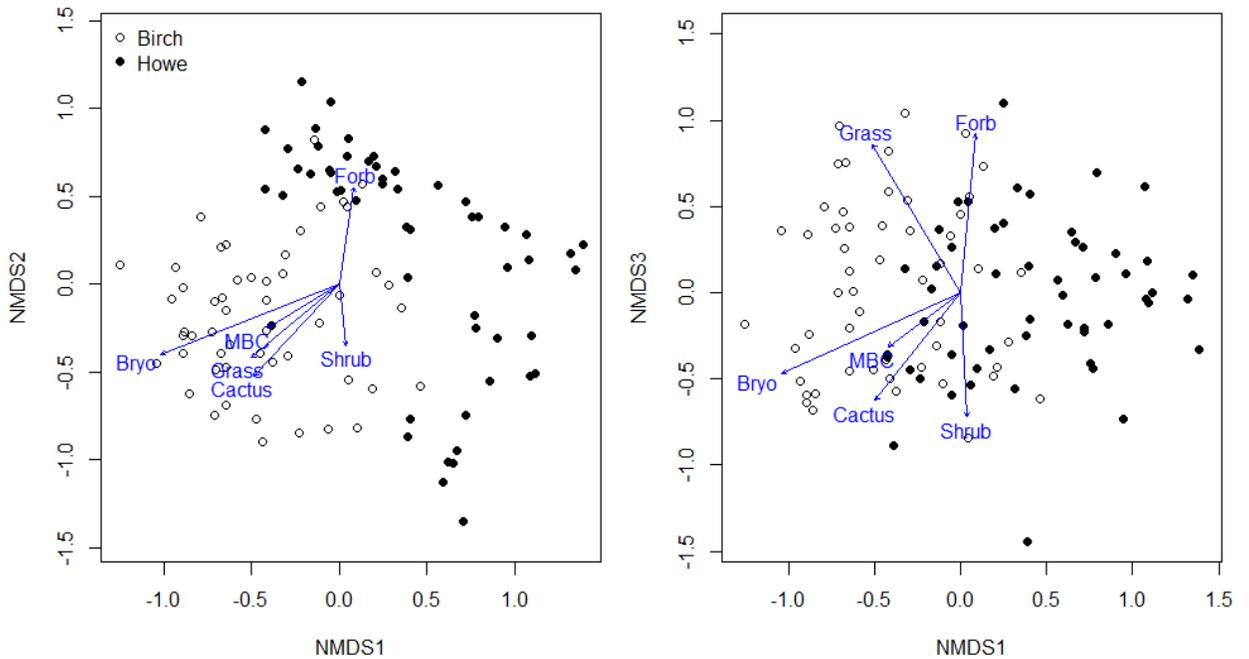


Figure 2. Graphs of NMDS analyses. Axis 1 shows Forbs and shrubs were slightly greater on the grazed site. Whereas all other classes had greater cover on the ungrazed site. Axis 2 shows that forbs were the only class that had greater cover on the grazed site. Axis 3 supports the greater diversity of grasses and shrubs in the grazed site. Idaho National Laboratory, Idaho, USA, summer of 2015.

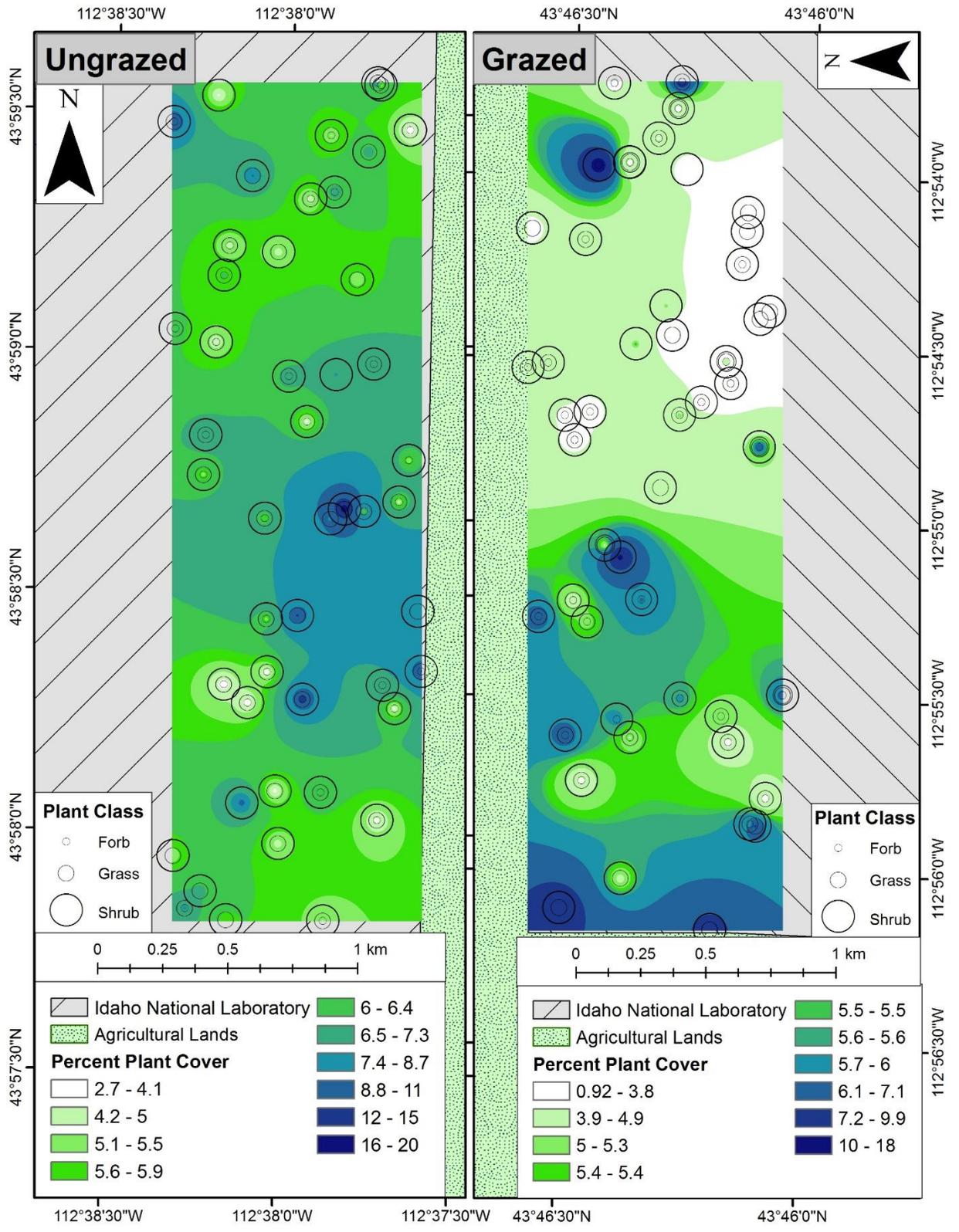


Figure 3. IDW map of plant cover. Water tank for cattle is 2km south of the SE corner of the grazed site. Idaho National Laboratory, Idaho, USA, summer of 2015.

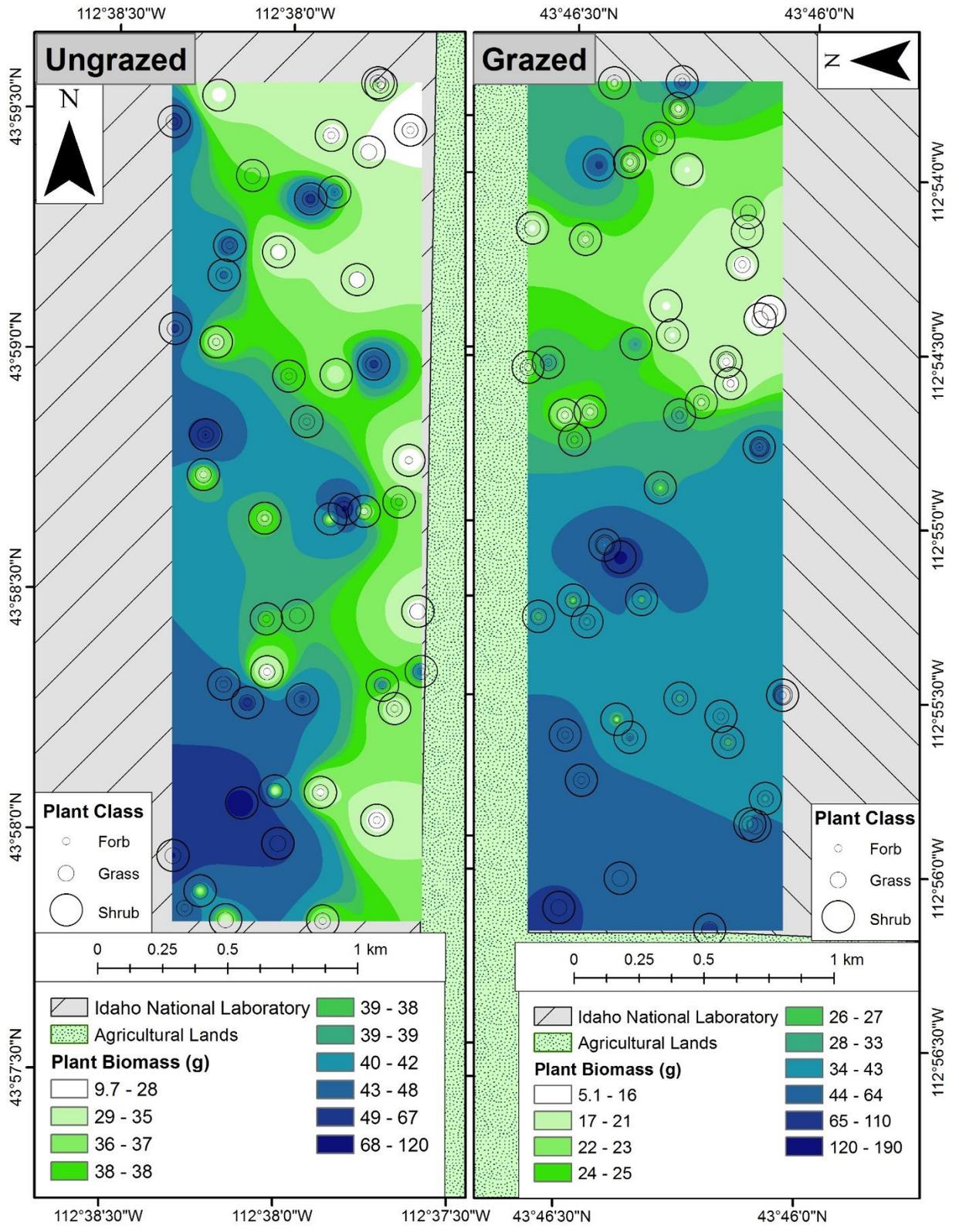


Figure 4. IDW map of plant biomass. Water tank for cattle is 2ckm south of the SE corner of the grazed site. Idaho National Laboratory, Idaho, USA, summer of 2015.