Project Completion Report
Rocky Mountains Cooperative Ecosystem Studies Unit (RM-CESU)

Project Title: Plant community effects on alpine ecosystem response to nitrogen deposition

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<u>Project Summary</u>, including descriptions of products, work accomplished and/or major results. If the information is restricted (e.g. location of endangered species or cultural resources), indicate the title and location of the final report. Also add web sites where project-related information may be found.

Research conducted under this project is in preparation for publication in peer reviewed journals for three main topics associated with the objectives of the original proposal: 1) Response of alpine moist meadow communities to nitrogen deposition, 2) Inter-annual variability in responses of alpine moist meadows: interactions of nitrogen and weather, and 3) Comparing responses of alpine meadow communities to nitrogen deposition: plant and soil processing of nitrogen. Data analysis associated with the first topic is almost completed, and is the focus of an accepted talk at the spring 2015 Rocky Mountain National Park Research Symposium. We are in the process of finalizing sample preparation and analysis for topics 2 and 3, although all sample collection has been completed. Below we include a brief outline of the main research questions, accomplished methods, and results associated with each of these topics.

Topic 1) Alpine moist meadow response to regional gradients of nitrogen deposition in the Rocky Mountains

Authors: Amber C. Churchill, Matthew J. Ribarich*, William D. Bowman
* Undergraduate student who worked three years on this project

Questions:

1. Are there ecosystem responses to nitrogen deposition along an ambient gradient in the Rocky Mountains?
2. Do plant-soil feedbacks change with nitrogen deposition along an ambient gradient in the Rocky Mountains?

Site Description and Experimental Design

To explore alpine responses to N deposition we established five sites located along a potential gradient of N deposition within the Southern and Central Rocky Mountains. All research sites were located in moist meadow alpine areas (higher than 3000 m; no trees or canopy > 1 m), with similar soil development (cryumbrepts derived from granitoid parent material). All sites had flat to < 15% slopes, and included presence of the main two dominant moist meadow plant species (*Geum rossi* and *Deschampsia caespitosa*). Sites we established in 2012 included Niwot Ridge LTER (Niwot; Colorado), Rocky Mountain National Park (ROMO; Colorado), and Shoshone National Forest (Shoshone; Wyoming). Sites we established in 2013 included Fraser Experimental Forest (Fraser; Colorado) and Arapaho National Forest (Arapaho; Colorado). At ROMO plots were established on Mt. Ida, Shoshone plots were located in the Grey Bull Ranger District, plots at Fraser were located in the Fool Creek Watershed, and plots at Arapaho were located on Kelso Mountain. All sites, with the exception of Fraser as this was located on the western slope of the Rocky Mountains, had an eastern aspect. All sites had three blocks established in separate moist meadow communities, with the number of plots among blocks constant for different types of measurements, as described below, to allow co-location of measurements. All plots we characterized for elevation, latitude and longitude using a handheld GPS (Garmin etrex 30).

Main Methods: Site scale analyses

We compiled thirty year mean monthly, and annual, precipitation records based on site coordinates using the PRISM model, as well as monthly minimum and maximum temperature (**Table 1**). These records we compared with long term records for meteorological stations present at a subset of sites (Niwot, ROMO, Fraser, Arapaho) to determine potential differences in climate among the selected sites, and possible deviations from the PRISM model estimates for higher elevation areas.

We measured growing season deposition of N (June 1- August 30th; scaled to 92 days) using passive ion-exchange resin columns installed at each site (Bytnerowicz et al., 2001; Fenn et al., 2009). Columns were attached to funnels for collecting rain and dust deposition in the field. Each column contained approximately 50 mL of mixed bed exchange resin beads (IONAC NM-60 H^+/OH^- Form; J.T. Baker), which we charged using 0.5 M NaCl prior to deployment. We also calculated absorption and extraction efficiencies for each batch of resin. During the summer of 2012 we installed 5-15 deposition collectors dispersed across each site to examine potential variation within a site; however elk damage reduced the final replicates. For 2013 and 2014 we installed 5 collectors immediately adjacent to each other, in a dry meadow community adjacent to the moist meadow blocks of interest at each site. During installation we attached each collector to 0.60 m PVC pipes inserted into the ground by 0.20 m for stability. After deployment and collection we maintained resin columns at 0 degrees C until extraction using 2M KCl solution. KCl extracts were then analyzed for concentrations of NO_3^-, NO_2^- and NH_4^+

using a Lachat QuikChem 8000 Specto- photometric Flow Injection Analyzer and a Dionex DX 500 System IonPac AS11 Ion Chromatograph (Kiowa Lab, INSTAAR, University of Colorado Boulder; Bowman et al., 2012).

As a means of confirming potential differences in bedrock chemistry, we collected representative rock samples from each site for determining concentrations of base cations in the bedrock. These samples we prepared using Whole Rock Analysis for composition of elements in each samples (IPC-OES analysis, Laboratory for Environmental and Geological Studies, University of Colorado Boulder). Additionally, for characterizing potential differences in soil qualities among the different locations, we collected soil samples within each block (n = 3 for each site) for soil texture analysis. These samples were analyzed for texture using a hydrometer for particle size analysis (Soil, Water, and Plant Testing Laboratory; Colorado State University).

Main methods: Measured ecosystem responses

We collected soil water using vacutainers attached to microlysimeters installed in five plots in all three blocks at a site for both 2013 and 2014 (n = 75 total; Rhizon soil moisture samplers, Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands). We sampled soil water during three phenological phases including post snow melt, during peak growing season, and after plant senescence (Bowman et al., 2012). Soil water samples were analyzed for concentrations of NO_3^-, and NO_2^- as described above.

For measuring soil available N, we constructed ion exchange resin bags using plastic cylinders (1 cm height). These bags we inserted at a depth of 10 cm, within the rooting zone, in five plots in all three blocks at a site for both 2013 and 2014 (n = 75 total per year). Resin bags remained in the field for the duration of the growing season (scaled to 91 days), and upon removal we stored samples at 0 degree C until extraction with 2M KCl. Adsorption and extraction efficiencies for each batch of resin we determined following the protocol above.

We collected bulk soils samples to co-occur with plots for vegetation surveys, resin bags and microlysimeters. These soil samples allowed us to determine within site variation in pH, cation exchange capacity (CEC) and carbon (C): N ratios. Samples included only the A horizon, to a depth of 15 cm, with a total volume of 1500 cm^3. Soils at Niwot, ROMO, and Shoshone we collected in 2012, soils at Arapaho and one block at Fraser we collected in 2013, and the remaining Fraser samples we collected in 2014. All soils we air dried in the laboratory and then sieved to a 2 mm fraction before subsampling for separate analyses. To measure soil pH we used a Beckman 340 pH probe on a 2:1 ratio of distilled water to soil paste determined after 30 seconds of shaking for five plots in each block at each site (n = 75 samples total). Another subset of soil we extracted with 0.1 $BaCl_2$, and analyzed the extractant for Ca^{2+}, K^+, Mg^{2+}, Na^+, Mn^{2+}, Al^{3+}, Fe^{3+} using an ARL Inductively Coupled Plasma Emission Specrophotometer (ICP-AES; LEGS, University of Colorado Boulder), for the same five plots in each block at each site.

The final subsample we homogenized using a ball mill and analyzed for C and N concentration using a CHN autoanalyzer (FLASH 1112 Series, Kiowa Chemistry Laboratory, INSTAAR, University of Colorado Boulder). These analyses included subsamples from 20 plots in each block at Niwot, 15 plots in each block at Shosone (block C has 20), 10 plots in each block at ROMO and block A at Arapaho, and 5 plots in blocks B and C at Arapaho. We also collected soils from 5 plots per block at Fraser.

We measured the composition of vascular plants species in 2012 (Niwot, ROMO, Shoshone) and 2013 (all sites) using a point-intercept method with a 10 by 10 grid of 100 points in each plot (vegetation plots were 1 m by 1 m; Bowman *et al.*, 2012). We measured twenty plots in each block at Niwot (2012, 2013), ROMO (2012, 2013), Shoshone (2012), and Arapaho (2013). At Fraser we measured a total of twenty plots (2013), and at Shoshone in 2013 we measured 10 plots in each block. Species nomenclature followed Weber (1976). Plant species that were present within a plot but not recorded at one of the points we assigned a projected cover value of 0.01. Due to a possible leaf area index higher than 1, total vegetation cover within a plot could be greater than 100%.

To measure net primary productivity (NPP) we harvested all aboveground vascular growth during mid-summer, at peak biomass, during the summer of 2014 in one 20 cm by 20 cm subplot for each of 10 plots per block at all five sites. These biomass harvests were sorted by hand in the laboratory to separate out new growth from litter, and then additionally sorted the two co-dominant plant species (*Deschampsia caespitosa* and *Geum rossii*), as well as sorting the remaining new growth into functional groups (grass, forb, shrub, sedge).

We collected specimens of each of the moist meadow co-dominant species (*D. caespitosa*, *G. rossii*) from 10 plots in each block at peak biomass, to determine the amount of C and N present in the growing plant tissues, in 2013 (all sites). We also collected specimens of each dominant species within these same plots following senescence to estimate potential plant N input to the soil at the end of the growing season. All specimens were oven dried at 60 °C, and then homogenized using liquid N, and a mortar and pestle. All samples were then analyzed for C and N using an elemental analyzer as described above.

Main results (selected and in progress):

Results from this study have shown that areas receiving higher levels of N deposition show increased concentrations of nitrate [NO_3^-] in soil water, and decreased cation exchange capacity and pH in moist meadow soils (Table 1). Additionally, there are significant differences in species composition among the five sites (Figure 1; MRPP; A = 0.3, all p values < 0.001), with key changes in the relative abundance of the two dominant moist meadow plant species (*D. caespitosa* and *G. rossii*). Future analysis will examine the influence of local climate and soil properties on site based differences relative to N deposition. Analyses to date have determined that changes in ecosystem processes among sites can be detected even at relatively low levels of ambient N deposition.

Table 1. Comparison of soil measurements by research site for samples collected 2012-2013

Measured N dep. (kg N ha^{-1} 92 $days^{-1}$)	Site ID	Soil water [NO_3^- and NO_2^-] (mgN/L) Post snow melt	Cation exchange capacity ($cmol_c$/kg)	Soil pH
1.0 ± 0.08^{ab}	Arapaho NF	0.44 ± 0.03^{a}	9.6 ± 0.4^{bc}	4.1 ± 0.04^{bc}
1.1 ± 0.10^{a}	Niwot Ridge LTER	0.19 ± 0.05^{ab}	10.4 ± 0.4^{bc}	4.2 ± 0.06^{bc}
0.75 ± 0.06^{b}	Rocky Mountain NP	0.20 ± 0.06^{ab}	7.9 ± 0.6^{c}	4.0 ± 0.04^{c}
0.80 ± 0.07^{ab}	Fraser EF	0.16 ± 0.03^{b}	13.8 ± 1.6^{b}	4.5 ± 0.10^{b}
0.66 ± 0.08^{b}	Shoshone NF	NA	21.5 ± 1.1^{a}	5.3 ± 0.08^{a}

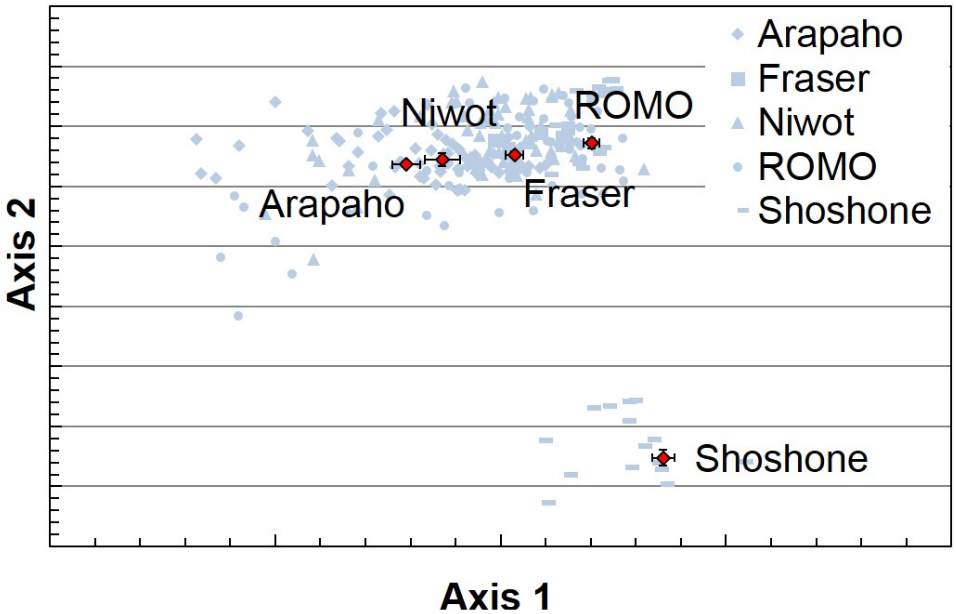

Figure 1. Non-metric dimensional scaling ordination of plant community composition among all sites. Final stress for 2-D solution was 12.63, with an instability of less than 0.0001. Mean ordination scores are shown in red, with SE bars shown for both axes.

Topic 2) Inter-annual variability in responses of alpine moist meadows: interactions of nitrogen and weather
Authors: Amber C. Churchill, William D. Bowman

Questions:
How does change in winter precipitation alter ecosystem response to N deposition?
Does variation in annual weather affect plant-soil interactions in response to N deposition?

Site Description and Experimental Design

This publication will use the same five sites outlined under Topic 1, with recent weather data gathered from meteorological stations in proximity to site locations (Niwot Ridge LTER saddle site, Fraser Experimental Forest alpine site, Loch Vale NADP site, and Mt. Evans), as well as recent estimates of N deposition associated with each of those sites, and snow chemistry data from the SNOTEL surveys (2011-2014). We will then use these weather and N deposition estimates in coordination with our measured summer N deposition to examine factors contributing to inter-annual variability in the ecosystem processes of alpine moist meadow communities responding to N deposition.

Main methods: Measured ecosystem responses

In addition to the collections and measurements described under Topic 1, we will also analyze dominant plant species biomass (collected during peak biomass) and litter (collected following plant senescence) C:N from samples collected in 2012, 2013 and 2014. For statistical analyses associated with Topic 2, we will therefore examine factors contributing to annual differences in soil N availability (2013, 2014), soil water [NO_3^-] (2013, 2014), plant community composition (2012, 2013), dominant plant biomass C:N (2012-2014), and dominant litter C:N (2012-2014).

Results

At present, we have analyzed samples for soil N availability, as well as soil water [NO_3^-], and there appear to be clear differences in these values among many sites between 2013 and 2014. The concentrations of soil water NO_3^-, in general, appear to be lower in 2014 than 2013 for all sites except Fraser Experimental Forest (Figure 2) for soil water samples collected immediately following snow melt. Soil N availability, an integrated measurement of N in the soil during the growing season, shows greater N availability for all sites except ROMO in 2014 as compared to 2013 (Figure 3).

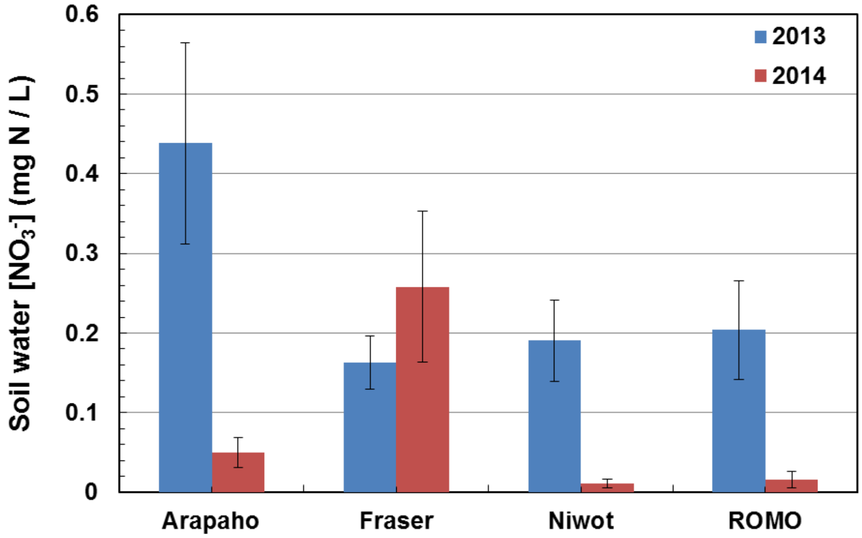

Figure 2. Concentration of nitrate in soil water samples collected using microlysimeters immediately following snow melt in 2013 and 2014. Bars are means ± SE.

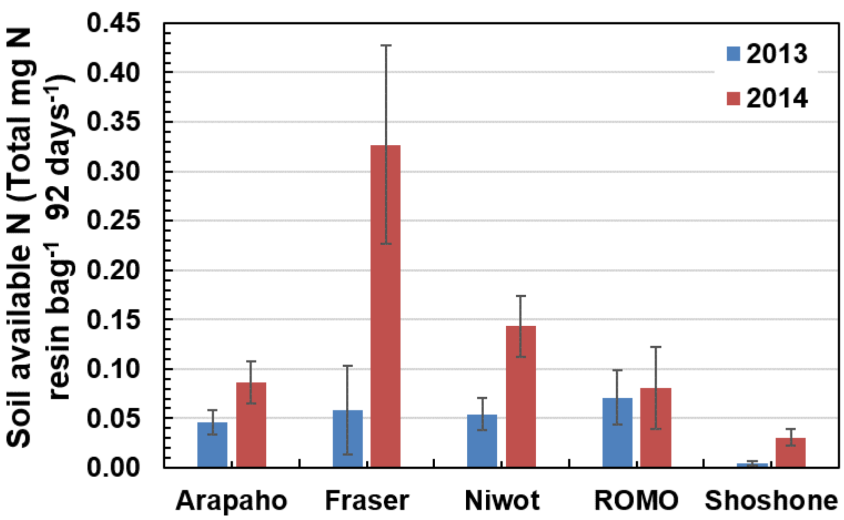

Figure 3. Soil N availability measured using resin bags in the plant rooting zone for the summer of 2013 and 2014 (scaled to a 92 day deployment). Bars are means ± SE.

Topic 3) Comparing responses of alpine meadow communities to nitrogen deposition: plant and soil processing of nitrogen

Authors: Amber C. Churchill, William D. Bowman

Questions:

How are ecosystem processes different among alpine communities, including dry, moist and wet meadows?

Which environmental or biotic driver is the greatest predictor of ecosystem processes among dry/moist/wet alpine communities?

Site Description and Experimental Design

This topic uses a subset of the sites outlined under Topic 1, including Niwot and ROMO, where three blocks of measurement plots were established in dry, moist, and wet meadow alpine communities in the summer of 2012. Site and block scale measurements were as described under Topic 1, including soil texture analysis for dry and wet meadow communities.

Main methods: Measured environmental and biotic drivers, and ecosystem processes

Environmental drivers we are examining for potential predictive power on ecosystem processes include measurements of N deposition, climate, soil texture, and soil C:N. We are considering estimates of annual N deposition associated with each site from local NADP stations, as well as summer measurements of N deposition collected under Topics 1 and 2, and winter snow chemistry data as potential drivers. Climate drivers are also associated with local meteorological stations (Topic 1). Soil samples associated with determining soil texture were collected from the A horizon during the summer of 2014, and analyzed as described under Topic 1. Soil samples associated with determining individual plot C:N ratios were collected in the summer of 2012, and included 20 plots per block at Niwot (for all three meadow communities), 20 plots per block for wet meadows at ROMO, and 10 plots per block for dry and moist meadows at ROMO. These soil samples were co-located with plots containing measurements of ecosystem processes described below, and all samples were processed for chemical analysis as described under Topic 1.

Biotic drivers we are considering are N storage in aboveground plants, including contributions from dominant and sub-dominant plant species, and plant community composition. We collected samples of aboveground biomass in ten plots for each block for all three community types during the summer of 2014. These samples are being sorted to determine NPP, as well as total contributions by dominant and sub-dominant plants as described under Topic 1. Plant categories from this harvest will be further processed to determine C:N ratios, as described under Topic 1, to determine aboveground stocks of N in plant material. Plant community composition was measured for 20 plots in each block for all three community types in the summer of 2012.

Ecosystem processes that we are comparing among communities include soil N availability using resin bags, and measurements of the concentration of soil water [NO_3^-]. Resin bags were installed in five plots in each block for all three community types in the summer of 2013, and N availability was determined as described under Topic 1. Soil water samples associated with [NO_3^-] were collected from the same five plots in each block for all three community types in the summer of 2013, for three sampling periods including immediately following snow melt, at peak biomass, and following plant senescence. Samples were processed for chemical analysis as described in Topic 1.

Results:

While many of the samples associated with this topic are still in preparation, we do have samples analyzed for soil N availability among the three community types. In general, it appears that the type of alpine community (and therefore associated predictors such as soil texture and soil C:N) is likely much more important than the particular site (and therefore associated climate or N deposition) in predicting soil N availability (Figure 4).

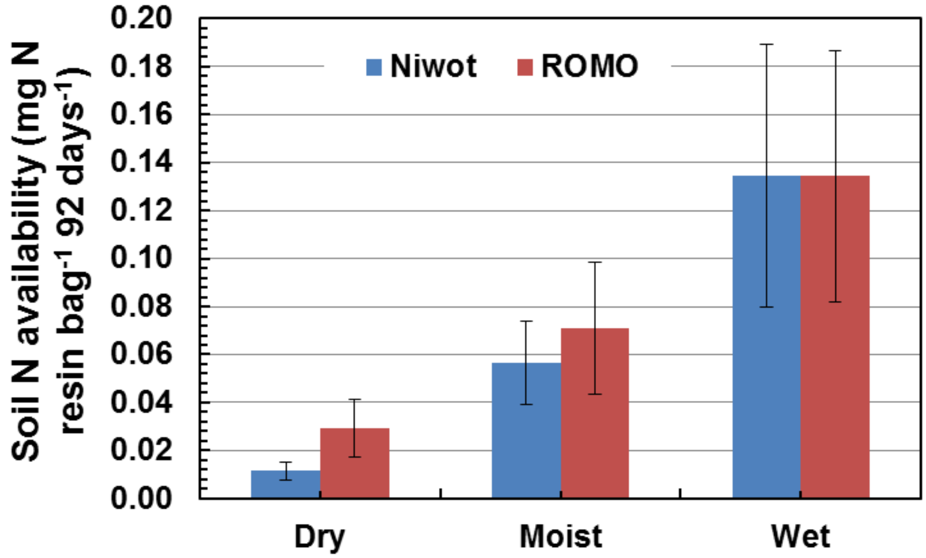

Figure 4. Mean soil N availability for alpine meadow communities measured during the summer of 2013 (error bars indicate standard error).

Number of students participating in this project: undergraduates, graduate students, degrees conferred.

Graduate students: Amber Churchill (1), PhD in progress

Undergraduate students: 10, two independent research projects for students who have graduated (BA in Ecology and Evolutionary Biology)

Lessons Learned from this project:

This project provided invaluable field training for 10 undergraduate students in measurements associated plant and ecosystem ecology. Additionally, the graduate student and one of the undergraduate students were able to share knowledge of the ROMO alpine flora with park visitors during the 2012 BioBlitz event.

From a research standpoint, initial efforts associated with this project have now expanded to form three independent subjects in preparation for publication, which will also be components of Amber Churchill's PhD dissertation research. While implications associated with the results from this study are still being developed, initial analyses do suggest differences in ecosystem processes associated with N deposition among the sites examined- supporting our hypothesis that even low level N deposition is sufficient to produce ecological effects in alpine environments.

Other RM-CESU agencies or research partners who participated in this project:

None.

Works Cited

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