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7 T.R. Seastedt^{*,1}, C.T. White^{*,1}, C. Tucker², E.M. Beaury^{1,3}, A.L. Concilio^{1,4}, E.I. Gasarch¹, V.J.
8 Haggans^{1,5}, and J.G. Smith¹
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13 * These authors contributed equally to the manuscript
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15 Author affiliations:

16 1. Department of Ecology and Evolutionary Biology and Institute of Arctic and Alpine
17 Research (INSTAAR), University of Colorado, Boulder Colorado USA

18 2. US Forest Service Northern Research Station, 410 MacInnes Dr., Houghton, MI 49931I

19 3. Department of Biology, Dartmouth College, Hanover, NH 037554.

20 4. Program in Environmental Science and Policy, St. Edward's University, 3001 S Congress
21 Ave Austin TX 78704 .

22 5. Department of Environmental Conservation, University of Massachusetts, Amherst, MA,

23 01003

24

25

26 Corresponding author timothy.seastedt@colorado.edu

27 ORCHID ID of corresponding author: 0000-0001-5079-7511

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Abstract

Plants often exhibit positive responses to multiple nutrient additions, but generalities about the life form and traits of the most responsive species are few. Findings from long-term experiments in dry meadow alpine tundra in Colorado, USA were used here to examine univariate and multivariate analyses of plant and plant trait responses. We found transient responses for community richness-biomass relationships, but a consistent increase in forb dominance in nitrogen (N) and phosphorus (P) addition plots. The response seen in the N+P plots was not affected by additions of micronutrients or potassium. Multivariate analyses corroborate transient responses in community shifts over shorter time scales, and directional shifts in N+P over two decades. Using species-level aboveground trait data when available, we found N+P may favor species with resource-acquisitive traits, regardless of their lifeform, as well as relatively tall grasses. In the absence of N and P limitation but with the environmental constraints found in dry alpine meadows, plant richness and community structure continued to exhibit transient states for decadal time frames.

Keywords alpine, community dynamics, graminoids, forbs, nitrogen, phosphorus

Introduction

While the causal relationships between plant diversity and productivity at various spatial scales remain contentious (e.g., Pillai and Gouhier 2019), our understanding of how resource abundance structures communities has greater potential for generality. This is, at least in part,

because of findings reported by scientists in the international Nutrient Network that have summarized patterns of production and plant species composition responses across climatic and nutrient gradients (c.f., Stokstad 2011). The abundance of soil nutrients, either as individual ions or in combination with others, frequently limit plant growth. For example, Fay et al. (2015) demonstrated that multiple herbaceous sites exhibited peak biomass production when released from nitrogen (N) and phosphorus (P) limitations, and showed that additional responses in growth sometimes occurred when potassium (K) and micronutrients were added. The enhanced productivity at these sites is the result of one or more species greatly increasing in size and abundance, often at the expense of slow-growing species or species adapted to low nutrient conditions. Depending upon the initial fertility of these sites, a linear or curvilinear pattern of decreasing species richness and diversity has been commonly observed with increasing productivity (e.g., Gough et al. 2000; Suding et al. 2005).

In the absence of aggressive, non-native plant species or extensive herbivory, graminoids (grasses, sedges, or rushes) are believed to be the common beneficiaries of most nutrient enrichments of herbaceous plant communities. While most forbs (non-woody dicots and members of the Family Amaryllidaceae) also respond to nutrient additions, the propensity for the grasses to grow taller than forbs and thereby generate light limitations to shorter-statured plants appears to give them a competitive edge in many communities. Arctic and alpine regions, however, represent a biome where temperatures and wind can penalize tall-statured plants. And, while graminoids are the recognized dominant life forms in both the arctic and alpine region in terms of cover (Billings and Mooney 1968), forb-dominated communities within these regions can be found (Chambers 1995; Winkler et al. 2016). In spite of the fact that forbs usually compose the minority percentage of vegetation cover, their abundance in the alpine is such that

we rarely call these communities ‘grasslands’ or ‘sedgeland’, but instead are ‘meadows’. Understanding why alpine regions in particular exhibit more intense floristic displays by dicots over more graminoid-dominated areas is a partially unresolved question. In addition to the impacts of soil resources, consumers such as gophers can also alter the relative abundance of these plant growth forms (Sherrod et al. 2005), and the year-to-year variation in floral displays appears to be an interaction of climate, resources, and individual plant species reproductive strategies (Galen 1990; Galen and Stanton 1993).

While most nutrient studies have focused on the response to N additions, sufficient N and P addition studies provide some insights into plant responses to these nutrients alone and in combination. High elevation and high latitude herbaceous plant communities appear to be more sensitive to P additions than other grasslands (Soons et al. 2017), perhaps due to energy constraints on mycorrhizae associations that provide P to most plants (Theodose and Bowman 1997). Of considerable importance, the moisture status of the plant community appears to be a major factor in determining the winners to N, P, and other nutrient additions. At several moist alpine meadows on the Tibetan Plateau in China, a series of studies by Ren et al. (2010), Lui et al. (2012), and Li et al. (2015) reported a strong response of grasses to N+P additions for up to eight years of treatment. Those studies reported that forbs exhibited either no responses or negative responses to N+P treatment, and that grasses obtained heights approaching 1.0 m in fertilized plots versus less than 0.6 m in controls. These plants are substantially taller than those observed in the Colorado alpine (Spasojevic and Weber 2018). At a Colorado site in the US, Theodose and Bowman (1997) reported that forbs and grasses exhibited equal cover status after five years of fertilization with N and P in dry meadows, but graminoids dominated responses of N+P plots in wetter meadows. At sites nearby the Theodose and Bowman study areas, Seastedt

and Vacarro (2001) reported equal dominance between grasses and forbs in control plots found in dry meadows, while modest forb dominance was observed in N+P plots after four years of treatment. As in all other alpine reports, graminoid dominance was observed in wetter meadows. Using these same plots with 19 years of results, Gasarch and Seastedt (2015) reported substantially increased dominance by forbs on the dry meadow sites. Based on these findings, one can conclude that site or moisture related effects in the alpine appear to result in context-specific findings in terms of forb or graminoid response to N+P additions, but that some consistent trends are apparent. At the same time, the extent to which findings might be transitory and represent an incomplete assessment of nutrient limitation effects on local plant species composition merits consideration.

The present study undertook multiple objectives to clarify and expand upon earlier work conducted on plant nutrient responses in the Colorado alpine. First, we asked if nutrients other than N or P affected plant species or lifeform abundances, a question addressed elsewhere but not at our site. In particular, Fay et al. (2015) noted that the limitation effect of potassium (K) and micronutrients alone or in combination with N and P affected productivity at 21 of 42 herbaceous (mostly grassland) sites. Second, we asked if the shifts observed in species and life forms in response to resource additions were consistent across space (i.e., in multiple dry meadows) and time at our alpine site. Third, we asked if the trade-off between alpine community production and diversity of local sites was consistent with previous reports within and across sites, and if transient responses affected the nutrient-diversity outcomes as well as the nutrient-trait responses (Niu et al. 2014). Spasojevic and Suding (2012) found functional traits associated with fast growth and resource-acquisitive strategy corresponded with resource availability along an environmental stress gradient near our site. Here we used dry meadow plant

trait data from Spasojevic and Weber (2018) collected near our sites to assess the extent to which functional traits explain species and community response to nutrient addition within moisture-limited sites. Collectively, these studies provide greater understanding of nutrient limitations in the alpine and evaluate the generality of findings from relatively short-term experiments.

Study Site and Methods

Research was done at Niwot Ridge, Colorado, located at 40.02° N, 105.35°W. This Long Term Ecological Research (LTER) site has been the focus of extensive research on multiple dimensions of terrestrial and aquatic high elevation research since 1980 (Bowman and Seastedt 2001, Williams et al. 2016). This alpine area occupies land between about 3100 to 4000 m above sea level (Bueno de Mesquita et al. 2018), with most studies located mid-range of this gradient. Vegetation descriptions as a function of the snow and moisture gradient generated by snow redistribution in the alpine are reported in Walker et al. (1993, 2001), Spasojevic et al. (2013), and Gasarch and Seastedt (2015). This vegetation has formed on a ridge consisting largely of Cryochrept soils not believed to have been disturbed by recent glaciation events (Burns 1980). Soil nutrient manipulative studies began with the dual purpose to understand the extent to which soil nutrient availability affected alpine species composition, but also to understand the impact of atmospheric deposition of N (Bowman and Steltzer 1998; Bowman et al. 2018).

Our research on plant response to nutrients began with a fertilization experiment initiated in 1993 reported by Seastedt and Vaccaro (2001) and then updated in Gasarch and Seastedt (2015). Here we focus only on the dry meadow plots used in that experiment. Sixteen 2 x 2 m

plots were established in an otherwise unmanipulated dry meadow region of an area known as ‘the Saddle’. Plots were randomly assigned one of four treatments (control, N, P, N+P) and fertilized accordingly. Rates and forms of N and P added to the plots changed slightly through time and details are found in Seastedt et al. (2019). Since 1998 10 g N m⁻² and 2 g P m⁻² were applied every other year and have continued to date. To buffer the soils from excess acidity caused by nitrification, amounts of N were added in the form of calcium nitrate since 2010, while P continued to be added as superphosphate. The dry meadow Saddle plots were inventoried for plant species cover in 2003, 2005 and again in 2016, with results included here with the previously published results from 1997 and 2012.

To address the possibility of multiple nutrient limitation including elements other than N and P, a second set of fertilization experiments in an adjacent watershed was established in 2007 using protocols similar to those of the Nutrient Network (NutNet 2019). The NutNet plots used here were initially surveyed and reported in a network-wide study (Adler et al. 2011), but have not otherwise been reported upon since treatments were established. Unlike other sites used in the Nutrient Network, no herbivory exclosures were added as these structures would have very strong effects on microclimate due to their propensity to accumulate snowpack. Four blocks of 10, 5 x 5 m plots were identified. Each block consisted of one replicate of each treatment and two replicates of the untreated control and ‘all nutrients’ plots.

Similar to the Saddle site, NutNet plots received 10 g m² N and 2 g m² P every other year beginning in 2008. Additional nutrients (Ca, Mg, S, B, Cu, Fe, Mo, Mn, and Zn, referred to here as micronutrients, ‘micro’) were added every other year beginning in 2008 at half of the rates used at other Nutrient Network sites (NutNet 2019). Treatments therefore consisted of eight levels of nutrient addition (control, N, P, micro, N+P, N+micro, P+micro, and N+P+micro).

Responses were assessed in a 2013 surveying effort. All of the previous treatments were applied again in 2014, but in 2016 potassium (K) was added as potash (K_2SO_4 , at $10 \text{ g m}^{-2} \text{ K}$) to the micronutrient plots while N and P was applied as before. Potassium was only added to the existing plots treated prior to 2016 with micronutrients. We refer to that treatment in the 2017 assessment as K+ plots.

Species competition and vegetation cover was conducted on all plots using point intercept procedures. At the Saddle site a gridded, 1 m^2 quadrat was used to generate 100 intersections, and 100 points were assessed for plant species presence. In 1997, only first (top) contacts with a vertical pin placed at the intercept were recorded, whereas in 2003 and thereafter any plant species that made contact with a vertical pin beneath the first contact were recorded at that point. These are summarized as ‘plant contacts.’ In 2005, only 50 contacts per plot were obtained, but both before and after that date 100 points were obtained per plot. Plant cover was estimated as the percentage of first contacts that struck vegetation as opposed to litter, rock or bare ground, and relative plant cover was the number of contacts of a single species divided by total vascular vegetation cover. Results reported here focus on vascular plant response, with *Selaginella densa*, a spike moss, included in this assessment.

The NutNet plots were assessed in the same manner, recording species and cover for 100 intercepts per plot. Plant diversity of in these plots was measured with the Shannon Index which included all species present within a 1 m^2 area. Species were given a score of 0.25 if present but not included in the cover estimates. Biomass was measured in 2013 by clipping 0.1 m^2 quadrats in each of the 40 plots, and weighed after drying biomass for three days at 70 C . Biomass was not measured in 2017. In 2013 and 2017 we also estimated the complexity of the canopy structure by analyzing total vegetation contacts per plot. This number reflects that there are often

multiple species occupying the canopy and provides an index of canopy complexity. Plots were sampled in July and early August in 2013 but in late June of 2017, a factor that may have affected results for cover and total plant contact results between years to a modest degree. All plots were sampled in 2013, but only 28 of 40 plots were sampled in 2017. In the 2017 sample, no plots receiving just P or P+K⁺ were included. Because there existed two replicates of control and N+P+K⁺ plots in each block compared with a single plot for all other treatments, the average values from the two replicates per block were used in all subsequent calculations. To ensure consistent comparison across time and sites, species identity data from the NutNet and Saddle sites were standardized to USDA PLANTS Database (USDA, NRCS 2019) species codes prior to analysis.

Statistical results were obtained by using a randomized factorial design for nutrient effects in the Saddle area, and a blocked, randomized design for the NutNet experiment using SAS 9.4 (SAS 2019). The complete sampling of all 40 NutNet plots in 2013 allowed for a full factorial analysis of N, P, micro effects, whereas the 2017 analysis used a one-way ANOVA to evaluate N, K⁺, N+P and N+P+K⁺ effects. When significant results were identified with the ANOVA, treatment differences were determined with a post-hoc SNK test.

We used Spasojevic and Weber's (2018) alpine functional traits dataset to describe resource strategies for species in our dataset and look for patterns in species ordination with nutrient treatment. Aboveground functional trait data at the leaf and organism level were collected for 137 species across Niwot Ridge from 2008-2018. We subsetted the dataset to data collected only in dry meadow communities in the Saddle, resulting in leaf trait variables for 14 species, 10 replicates per species, and all data collected in 2017. We selected 12 trait variables associated with resource strategy that had complete data: Leaf dry matter content (LDMC), C:N

ratio, overall plant height, leaf dry weight, leaf area, leaf mass area (LMA), percent leaf mass C, percent leaf mass N, specific leaf area (SLA), mean leaf chlorophyll content, and isotopic D13C and D15N. We log transformed height, dry weight, leaf area and SLA to achieve more normal distributions, calculated mean trait value per species, and centered and scaled all variables before running the PCA. Species in the Saddle and NutNet composition dataset that don't overlap with the subsetted trait dataset are designated as "unknown" for their resource strategy in results presented below.

Incorporating results from the PCA, we assessed nutrient addition effects on Saddle and NutNet community composition and shifts in forb:grass abundance through time using non-metric multidimensional scaling (NMDS), PERMANOVA, and testing homogeneity of dispersion based on Bray-Curtis community dissimilarity matrices. To minimize compositional shifts from changes in personnel and their botanical ability over time, we combined species that would be difficult to identify based on co-author expertise in botanical surveys at Niwot Ridge. We also excluded unknowns and any species that did not occur in at least 5% of the observations and sum to at least 10 hits by site to remove noise from rare species. NutNet and Saddle sites were analyzed separately. All multivariate analyses and supporting statistical tests were done in R (R Core Team 2019) using packages 'vegan' (Okansen et al. 2019).

Results

Multiple nutrient controls on alpine productivity and diversity

After four years of treatment, Seastedt and Vacarro (2001) reported that N+P additions to dry meadow Saddle plots increased productivity and decreased plant richness, a trend that continued

for the next 15 years (Gasarch and Seastedt, 2015). A repeat inventory of the 16 dry meadow plots four years following that study also found the same outcome (data not shown).

The findings from the 2013 inventory of the NutNet plots demonstrated that the additional nutrients added in the ‘micro’ treatment had no significant effects on alpine plant foliage biomass, richness, diversity and canopy complexity (Table S1). Further, there were no significant interactions with either N or P and the micronutrients. Accordingly, the results from micro plots have been pooled into control, N, P, or N+P plots to illustrate the significant findings observed five years after initial fertilization treatments (Table 1, S2). In contrast to the Saddle plots, these results indicate that N additions had a slightly stronger and positive effect on richness, cover, biomass, and vegetation contacts relative to P, but P also had generally positive effects on these variables as well. The combined N+P effect on richness and diversity appears additive, but effects on plant biomass and vegetation contacts appeared to be synergistic. The release of the co-limitation of N and P on plant growth more than doubled biomass of the control plots on these N+P plots, and the increase in canopy complexity (vegetation contacts) increased more than just the sum of the individual N and P plot increases.

Measurements of richness, diversity, and plant cover were repeated in 2017 on a portion of these plots after potassium sulfate was added to the micronutrient plots in 2016. Results of a one-way ANOVA demonstrated no effects of K + micronutrients on richness, diversity or cover, but a reduction in vegetation contacts in the K+ was found when compared to N+P+K plots (Table 2). The 2017 effects of N indicated a reduction in plant diversity of N plots compared to the N+P+K plots. Overall, N+P and N+P+K plots produced results similar to the Saddle responses, with no effect on richness or diversity compared to control or K+ plots. In particular and unlike 2013, positive effects of N and P on richness and diversity were not observed in 2017.

The vegetation canopy did, however, increase in complexity in multiple nutrient addition plots, which produced more total vegetation contacts than either the control or K+ plots (Table 2, $p<0.05$). Overall, there was a trend in increasing cover and canopy complexity in the N+P and N+P+K plots, consistent with responses seen in 2013.

Response of life form and selected species to multiple nutrient additions.

The relative cover of graminoids and forbs progressively changed through time in response to the combined additions of N and P. This is clearly evident when results from the Saddle plots surveyed after 4, 19, and 23 years of treatment are viewed together (Fig 1). Relative to controls, graminoids tend to respond to N additions while neither graminoids nor forbs appear to change in relative abundance in response to P additions. The results from the NutNet plots, obtained 5 and 9 years after the initial treatment demonstrated a similar trajectory for most treatments (Fig 1), but forb:graminoid ratios were not statistically different between years or among the treatments (data not shown).

The dominant forb responsible for much of the increase seen in the Saddle was a member of the rose family, *Geum rossii*, an abundant species found across dry to mesic environments at our site (Gasarch and Seastedt 2015). This species increasing dominance, particularly in Saddle plots, was measurable (Fig 1), but there also appears to be an increase in cover through time regardless of treatment (Table 3). The relative abundance of *Geum* at the Saddle site increased from 9.7% to 14.3% of cover in control plots over a 19 year interval. In NutNet plots, *Geum* averaged increases from 7% to 10% of cover in control plots in four years. In the Saddle plots, four years of N+P treatment suggested an increase from 12.9% to 23.5% of cover from 1997 to 2003, and this value increased to 40.2% of cover in the same plots after 23 years. In apparent contrast, *Geum* cover in the NutNet plots increased in general but was not statistically more

abundant in N+P plots when compared to control plots after 5 or 9 years (Table 3). A closer evaluation of species trends in NutNet plots revealed that the increasing dominance by forbs was accomplished by a combination of species other than the non-statistically significant increases in *G. rossii*, and also that several graminoid species also showed a positive response to N+P treatments, while the historically dominant species of graminoids declined in N+P plots (Table S3).

Trait PCA

Almost half (44%) of the variation in the aboveground dry meadow plant trait data can be explained by a gradient of resource and growth strategy and two groups of species separate along this first axis (Fig. 2). The variables that load the negative side of axis 1 (LMA, LDMC, C:N ratio and carbon as D13C) suggest slower growth, more retention of carbon, and slower decomposition rates. Variables that suggest resource acquisitive strategies, more investment in above-ground tissue, and faster nutrient cycling and decomposition (e.g., greater leaf chlorophyll content, leaf ANPP, SLA, leaf N, and overall height; e.g., Reich 2014) drove the positive side of axis 1. We therefore interpreted PC1 as a gradient of resource strategy and define species with a PC1 score of 0 or below as “resource conservative” and species with PC1 score greater than 0 as “resource acquisitive”. PC axis 2 accounts for an additional 18% of the variation in the data and is loaded most strongly by height in the positive direction. Species identities are shown in Fig. S1 and resource strategy, as available, identified for species in Table S3.

NMDS and multivariate analysis

Both Saddle and NutNet dry meadow sites show N+P additions resulted in floral communities distinct from communities in other treatments (Fig 3). Moreover, there was a significant temporal trend in N+P communities becoming increasingly dissimilar from other

nutrient treatments at the Saddle site. Plot-level communities fluctuated variably in composition (indicated by the length and direction of lines connecting plots in Fig 3A and 3b) between sampling visits on shorter-time scales (e.g. 4-year intervals), by treatment and by site, although control communities tended to fluctuate less at both sites, by plot and on average (treatment arrows in Fig 3B and 3D).

Qualitatively, NMDS species ordinations and patterns based on species resource strategy and lifeform suggest some similarities and differences between the dry meadow communities. For example, Fig 3B and 3D suggest stronger clustering by resource strategy at NutNet compared to the Saddle, although there are only 10–11 species described by the trait PCA at each site so we interpret this modestly. Ordinations also suggest *Geum rossii* associates with N+P treatments, although the overlap is more apparent at Saddle, and grass species also associate with N+P. For example, although *Deschampsia cespitosa* and *Festuca brachyphylla* are described as resource conservative by PC1, they align with N+P treatments at both sites (conservative-colored squares nearest N+P in Fig 3B and 3D), which may relate to a competitive advantage over forbs because of plant height.

Treatment and forb:grass abundances (lnF2G) are consistently significant factors in PERMANOVA at Nutnet and Saddle and account for similar variation in the data, controlling for temporal dependencies in observations ($p < 0.001$, Treatment R^2 approximately 30% at both sites and lnF2G 10% at Saddle and 18% at NutNet). Saddle is distinct from NutNet in that year, year x treatment, and year x forb:grass abundance are also significant in PERMANOVA ($p < 0.01$) and each accounts for about 5% of variation in the data. It is important to point out Saddle sites have several time points to test over a 23 year span, and NutNet has 2 time points over a 11 year span.

Homogeneity of variance in dispersion was similar at all sites, for all treatment-year combinations (betadisper n.s.).

Discussion

While alpine plant communities appear to almost always respond to N additions, these communities generally appear even more responsive to multiple nutrient additions. This is not a new finding (e.g., Wielgolaski 1975), and our work agrees with Soons et al. (2017) that cold region sites often exhibit both N and P limitations. The larger, combined N+P limitation as opposed to single element response appears to be the rule rather than the exception in herbaceous communities (Fay et al. 2015). The fact that our NutNet site exhibited greater than a doubling of biomass in response to N+P (Table 1) demonstrates the extent to which these elements can limit local productivity in the alpine environment.

Our experiment failed to demonstrate any nutrient limitations other than those imposed by N and P. A local finding, true for all dry meadows studied at our site, was that forbs can become dominant under high N+P conditions. This generality does not appear to hold for all cold regions. Madan et al. (2007) did not find life form a reliable predictor of plant response in the high arctic. That said, we predict that the forb life form under high N+P is often favored in cold region sites that have wind or water limitations, but our work also shows that responses to nutrient additions require time to appear. Diggle (2003) summarized how dicots exhibit conservative growth strategies in alpine environments that limit their ability to immediately respond to resource additions. Grasses appear to have more consistent opportunistic growth strategies, and grasses in alpine environments at multiple sites clearly do respond to N+P (Lui et al. 2012; Li et al. 2015). Here, we also noted several grass species in dry meadow environments

capable of responding to N+P (Theodose and Bowman 1997, Table 4), and those species, similar to some forbs, may continue to increase in relative dominance through time.

In addition to nutrient amendments, Seastedt and Vacarro (2001) measured plant nutrient responses to additional moisture via a snowfence manipulation. Plots initially classified as dry meadow sites prior to the addition of the fence were very strongly affected by snow additions. For example, *Geum rossii*, which averaged 8.2% of plant cover across dry meadow plots, averaged 24.1% plant cover in snow-amended plots four years after initiation of treatment. Since initial conditions in these plots was not assessed, we cannot be certain all differences were due to moisture change. However, Turner (2002) also monitored *G. rossii* response within the snowfence, and reported increases from 11.5% to 21.5% after seven growing seasons. Thus, the response of *G. rossii* to increased moisture appears to mimic that seen for its response to N+P additions.

A parallel wetting and warming experiment was conducted in an area adjacent to our sites while our studies were ongoing (Winkler et al. 2016). That site was much rockier than ours and would classify as a fellfield community based upon average plant composition of 23% cushion plants and succulents, 12% graminoids, and 65% forbs (Winkler et al. 2016). That study also reported both high values of *G. rossii* (37% relative cover), and that forbs rather than graminoids were very responsive over a four year interval to a warming and wetting treatment. That response was reversed, i.e., graminoids outperformed forbs, when warming was not accompanied by wetting. Since graminoids in that study were relatively short, low statured species, competition for light was, as in our study, not believed to be a factor affecting outcomes. The large and fairly rapid response of forbs seen in their study, combined with the snow addition

findings of Seastedt and Vacarro (2001) and Turner (2002) argue that moisture availability in the alpine is a strong driver of vegetation composition across a portion of the alpine landscape.

The sensitivity of *G. rossii* to moisture may also explain its differences in nutrient response observed here across the multiple site experiments. The failure for *G. rossii* to respond to N+P treatments in the Theodose and Bowman (1997) study may have been due to the relative short time of the experiment, but the longer duration study in the NutNet plots suggests other variables may also be responsible. Under water limitation *Geum rossii* is thought to grow rather slowly and appears to create low N soils by producing root exudates that enhance N immobilization (Meier and Bowman 2008). If viewed as a competitive mechanism, this exudate strategy should not be effective under large N additions, yet the species becomes more abundant through time under N+P additions. Perhaps the large taproots these species possess are better able to store carbohydrates such that growth is subsidized each year with last year's NPP (Körner 2003). A constant, positive subsidy to roots could result in exponential growth, as seen in the Saddle sites, and dominance through time. These larger roots might also improve water acquisition.

Forb dominance to N+P has also been observed in the North American grasslands. Avolio et al. (2014) reported that annual forbs replaced grasses and became dominant on upland (relatively dry) tallgrass prairie after about a decade of treatment. Annual forbs clearly could not use a storage accrual strategy to out-compete grasses as postulated for the alpine, indicating that multiple ways that allow forbs to assume dominance in herbaceous systems under nutrient enrichment.

The addition of N+P increases both vegetation cover and canopy complexity. The average total number of plant species per intercept point increased somewhat more than did

vegetation cover (Tables, 1, 2). Roberts (2018) showed that forb species in dry alpine meadows compose a relatively higher percentage of vegetation in the alpine sub-canopy than canopy (i.e., below the ‘first hit’ using point-intercept methods), and this finding adds a potential experimental artifact when comparing results among studies that have used different point-intercept procedures. In particular, our 1997 findings likely have a modest graminoid bias relative to subsequent studies because of the failure to record multiple vegetation contacts. That said, several alpine grass species did show a similar increase to N+P through time as did some forbs (Table S4). Hence, life form in the alpine, as seen elsewhere, is no guarantee of species success under a specific nutrient regime.

These long-term nutrient addition studies have the potential of adding insight to understanding mechanistic responses to warming or wetting/drying scenarios for the alpine (e.g., Winkler et al. 2016). If, for example, climate change alters nutrient availability such that plant responses are actually climate-induced nutrient responses, then our experiments may have the potential to identify this change by observing time series patterns of controls vs various nutrient additions, a finding which cannot be isolated by warming/drying experiments alone. The outcome of nutrient amendments to plant richness and diversity appears to almost universally indicate species reductions, and our work agrees with that finding provided sufficient time is allowed for the response to develop. The mechanisms responsible for successes (increased abundance) and failures (declines or local extirpations) of plant species clearly vary among sites, and in the alpine at least, changes in relative abundance continue after decades of continued treatment.

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List of Figures

Figure 1. Comparison of time trends for relative cover of forbs, graminoids, and *Geum rossii* across all treatments found on Saddle and NutNet plots on Niwot Ridge (means, ± 1 std error, $n = 4$ sites per treatment). The 2017 measurements did not include P plots.

Figure 2. Principal component analysis (PCA) of 10 trait variables for 14 species collected from the Saddle dry meadow communities. Light and dark colored points indicate where species fall on a spectrum of resource strategy (PC axis 1), with lighter colored points corresponding to species with resource acquisitive, fast-growth strategies and dark colored points resource conservative growth strategies. Species identities for colored points shown in Figure S1.

Figure 3. NutNet and Saddle dry meadow NMDS, showing plot shifts through time (left-side panels) and species ordinations with mean treatment shifts through time (right-side panels). Shaded nutrient treatment centroid 95% confidence ellipses ($n = 4$ sites per treatment) shown in all panels for the most recent year of survey by site. Envfit vectors for log-transformed forb to grass ratio and year shown as solid gray arrows with significance noted (right-side panels, $p < 0.001^{***}$, $p < 0.01^{**}$, $p < 0.05^{*}$, $p < 0.1$, $p > 0.1$ n.s.).

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612

613 Figure 1.

Figure 2.

Figure 3.

621 Table 1. Alpine plant community patterns found on Nutrient Network (NutNet) plots in 2013.
 622 Values are means and std. errors from 1 m² samples (means, std errors, n = 8 per pooled group).
 623 Statistical analysis for table reported in Table S2.

Variable	Treatments			
	C, C+micro	N, N+micro	P, P+micro	N+P, N+P+micro
Richness	22.6 (1.81)	25.2 (2.56)	23.9 (2.40)	27.1 (2.75)
Diversity	2.2 (0.11)	2.4 (0.16)	2.4 (0.14)	2.7 (0.09)
Plant Cover	86.6 (0.92)	94.8 (1.30)	87.9 (1.75)	97.8 (0.50)
Biomass	137.5 (18.5)	195.0 (14.9)	150.4 (4.75)	331.4 (26.04)
Vegetation contacts	134.8 (8.98)	168.3 (9.40)	142.4 (1.20)	214.5 (9.93)

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Table 2. Richness (# species per m²), Shannon Diversity, plant cover (vascular vegetation present/100 intercept points) and total vegetation contacts per m² for NutNet plots in 2017 (means, ± 1 std error, n=4 per treatment). Values followed by different letters are significantly different from other treatments (SNK post-hoc test, $p < 0.05$).

Variable	Treatment				
	C	K+	N	N+P	N+P+K+
Richness	20.8 (3.03) a	18.8 (0.64) a	18.5 (2.50) a	18.3 (2.05) a	22.1 (2.22) a
Diversity	2.3 (0.15) ab	2.1 (0.11) ab	1.8 (0.26) b	2.3 (0.18) ab	2.6 (0.03) a
Cover	77.1 (4.57) a	75.5 (5.20) a	87.8 (3.11) a	87.3 (5.65) a	86.4 (3.89) a
Vegetation contacts	105.5 (17.21) b	96.5 (11.38) b	123.8 (9.18) ab	125.3 (14.42) ab	143.1 (16.08) a

Table 3. Mean relative vegetation cover of *Geum rossii* at alpine dry meadow plots over time (means, ± 1 std error, n = 4 per treatment). Plots treated with P additions at the NutNet site were not sampled in 2017.

Treatment	Saddle Sites (1997-2016)					NutNet Sites (2013, 2017)	
	1997	2003	2005	2012	2016	2013	2017
C	9.7 (2.37)	19.23 (5.83)	13.5 (2.68)	12.77 (1.46)	14.32 (3.08)	6.99 (2.85)	9.95 (2.28)
N	7.16 (1.96)	19.85 (2.42)	22.83 (5.94)	10.87 (2.13)	18.07 (0.86)	5.53 (2.81)	10.96 (5.57)
P	12.52 (7.57)	8.08 (2.49)	6.41 (2.28)	8.66 (2.02)	8.4 (3.39)	9.69 (3.98)	-
N+P	12.88 (3.43)	23.5 (5.53)	23.54 (8.62)	30.59 (8.32)	40.21 (9.33)	4.67 (3.46)	13.36 (12.73)