PLANT COMMUNITY MEDIATED RESPONSES OF ALPINE ECOSYSTEMS TO ANTHROPOGENIC NITROGEN DEPOSITION

by

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Plant community mediated responses of alpine ecosystems to anthropogenic nitrogen deposition

Thesis directed by Professor William D. Bowman

Human alteration of the nitrogen (N) cycle has resulted in a rapid increase in the availability of biologically active N. An unintended consequence of increased N availability is increased levels of N deposition into natural landscapes, including alpine ecosystems which are particularly susceptible to adverse environmental impacts. In my dissertation, I have examined alpine plant and soil responses to N deposition 1) across multiple spatial scales throughout the Southern Rocky Mountains, 2) among diverse plant communities associated with unique environmental conditions common in the alpine of this region, and 3) among ecosystem pools of N contributing to stabilization of N inputs within those communities. I used a gradient of N deposition in the southern Rocky Mountains to examine spatial variation in the responses of an alpine moist meadow to gradual changes in N deposition. I developed a response framework of expected ecosystem changes associated with increasing inputs, and found that plant metrics, such as tissue concentrations of nitrogen, were positively correlated with ambient N inputs. Soil biogeochemical responses to N were minimal along the gradient, suggesting that a reduction in N inputs may allow ecosystem recovery if plant feedbacks to N cycling are negligible. I next established a N fertilization study in three common plant communities to compare community responses to simulated N deposition and examined the applicability of the response framework throughout the alpine. I found that communities responded to inputs of N differently for both plant and soil responses, with the dry meadow community showing the strongest effects of N inputs and at the lowest levels of N input while the wet meadow community had few responses contributing to ecosystem change. Finally, I compared the fate of N inputs among ecosystem pools of N within alpine communities receiving elevated N. I found differences in N uptake among the communities, and that uptake increased with elevated N inputs. Even so, ecosystem uptake of N in plant and soil pools was very low (7-11%), supporting the conclusion that the alpine ecosystem does not retain N inputs and that increased N deposition is unlikely to be stabilized in the alpine.

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CHAPTER 1
Introduction

We have entered the Anthropocene, an era associated with human driven alterations of geologic and biological functioning on Earth, surpassing key thresholds for irreversible modifications to biogeochemical cycling of carbon, nitrogen (N), and phosphorus (Rockström et al. 2009a, Steffen et al. 2015, Waters et al. 2016). In particular, human alteration of the N cycle has resulted in a rapid increase in the availability of biologically active N (Vitousek et al. 1997, Galloway et al. 2003, 2004). An unintended consequence of this change is distribution of reaction N through N deposition, a process is tightly coupled with weather and wind patterns across ecosystems. There are many impacts associated with increased N deposition, ranging from ecosystem scale changes such as altered processing of N and acidification of soil and surface water, to local scale changes in diversity of plants (Aber et al. 1998, Erisman et al. 2008, Bobbink et al. 2010). The magnitude of these impacts of N deposition can be affected by ecological feedbacks acting to promote or prevent changes through plant-soil interactions that can persist even after N deposition lessens. In my dissertation, I have sought to examine alpine plant and soil responses to N deposition on multiple spatial scales throughout the Southern Rocky Mountains, among diverse plant communities associated with unique environmental conditions, and between pools of N contributing to stabilization of N inputs within those communities.

Background information
Ecosystem responses to nitrogen deposition

Anthropogenic production of synthetic fertilizers and fossil fuel combustion have resulted in an increase of reactive N on a global scale (Vitousek et al. 1997, Galloway et al. 2008). This increase is facilitated by wet and dry deposition of both organic and inorganic forms of reactive N, with higher deposition in areas near agriculture (such as cattle feed lots) and industry (burning of fossil fuels) (Nanus et al. 2003). Increases in the both the amount of deposition of N and duration of additions have a number of well-established consequences for community structure and ecosystem function, although less is known about potential feedbacks between community and ecosystem processes. Community and ecosystem responses to elevated N deposition occur in a hierarchical framework, such that the response of some metrics can influence the potential for change in other metrics (Aber et al. 1989, Smith et al. 2009). Plant response metrics, such as litter chemistry, in particular have the potential to act as plant-soil feedbacks contributing to community-ecosystem interactions, while soil metrics indicate the cumulative influence of biotic and abiotic feedbacks (**Figure 1.1**).

As N accumulates in an ecosystem, the first plant responses are associated with physiological changes of individual plants such as increased primary production (Aber et al. 1998, Smith et al. 2009). Results associated with these responses are not frequently reported, although they have particularly important implications for plant-soil feedbacks and the potential for plant stabilization of N within an ecosystem (Templer et al. 2012). Following individual responses, increased inputs may be associated with shifts in the abundance of individuals within populations and changes in the strengths of interactions among community members. Generally, levels of N necessary to affect plant community composition are between 5-20 kg N ha^{-1} yr^{-1} (Bobbink et al. 2010), but some studies suggest compositional changes under N inputs as low as 3 kg N ha^{-1} yr^{-1}. Nitrogen inputs between 3-20 kg N ha^{-1} yr^{-1} are often lower than current ambient conditions for many regions around the world (Galloway et al. 2004), suggesting that plant biodiversity has already been impacted in some areas around the world (Suding et al. 2005, Bobbink et al. 2010, Simkin et al. 2016). Gradient studies of ambient deposition, in the United Kingdom and across European grasslands support this suggestion, and estimate potential species loss at N addition levels as low as 2.5 kg N ha^{-1} yr^{-1} (Stevens et al. 2004, Duprè et al. 2010). Additionally, numerous studies have documented a loss of species richness and diversity with experimental additions of N, ranging from short-term high dosages to chronic treatments with low levels of simulated deposition (Stevens et al. 2004, Suding et al. 2005, Bobbink et al. 2010, Clark et al. 2013). These changes have important implications for the long-term changes in plant-soil feedbacks and total retention of N by the plant community, and especially for the potential for the introductions of non-native species as rare native species are outcompeted under high N input conditions (Smith et al. 2009, Templer et al. 2012).

Increases in N inputs also have an effect on soil processes, including changes in rates of mineralization and nitrification that produce changes in the soil N pools (Aber et al. 1998, Gundersen 1998, Corre et al. 2010). Studies examining the influence of N deposition across North America and Europe have shown that deposition levels greater than 10-20 kg N ha^{-1} yr^{-1} promote the production of nitrate and contribute to significant leaching of nitrate from forest and alpine soils (Dise and Wright 1995, Fenn et al. 1998, Bowman et al. 2006). These increases in nitrate can leach soil cations lowering buffering capacity that, under extreme levels of N addition, result in soils and increases in soluble aluminum and manganese that are toxic to plants and microbes (Vitousek et al. 1997, Aber et al. 1998, Fenn et al. 2003a, Bowman et al. 2008).

Despite this general understanding of ecosystem responses to elevated N, less is known about spatial variation in responses associated with low-levels of N inputs as opposed to high dosage fertilization studies. This information is particularly important in building knowledge of ecosystem processing of N in early stages of the environmental impacts of N deposition in order to more fully understand how future change will affect at-risk regions. Despite the number of studies investigating ecosystem responses to increased inputs of N, less is known about the processes that can either promote or reduce ecosystem change as measured by different response metrics associated with the long-term effects of N deposition (Chapin et al. 1997). These processes include plant influence on N cycling through differences in feedbacks and pools of N that may stabilize N among different plant communities, ultimately altering the potential for ecosystem responses.

Plant controls on ecosystem response to N deposition

There are many in-direct feedbacks between plants and ecosystem responses to N deposition associated with plant driven fluxes of the N cycle. While these fluxes are primarily associated with individual plants, or plant species, it is the net contribution of species as a community that comprise potential amplifying or stability feedbacks associated with ecosystem change under elevated N (**Figure 1.1**). Thus, changes in the plant responses among communities may have important future implications for the long-term sensitivity of different ecosystems to continued inputs of N.

One mechanism that allows plants to influence ecosystem processing of N is through species-level variation in N uptake (**Figure 1.1**; Plant N uptake from soil; green arrow). This occurs through both total uptake in the amount of N and species-specific preferences in uptake of N type from the soil, as there is evidence for species favoring either nitrate or ammonium or even organic forms of N (Hobbie 1992, Suding et al. 2004, Ashton et al. 2008). Plant control of the abundance of different forms of N may then contribute to ecosystem microbial immobilization and nitrate leaching, acting to promote or prevent biogeochemical ecosystem responses to N deposition (Buchmann et al. 1996, Miller and Bowman 2002, Ashton et al. 2008, Corre et al. 2010, Darrouzet-Nardi et al. 2012). Nitrogen taken up from soil can be transported from roots into aboveground tissues (Chapin 1990). For most plants, this uptake of N often translates directly into new growth or net primary productivity (NPP) (Smith et al. 2009). However, in the physiologically constrained alpine flora, luxury uptake and increased tissue concentrations of N may occur (Billings 1974, Bowman 1994, Lipson et al. 1996, Meloche and Diggle 2001, Korner 2003). Plant retention of N after incorporation into tissues varies temporally, from seasonal

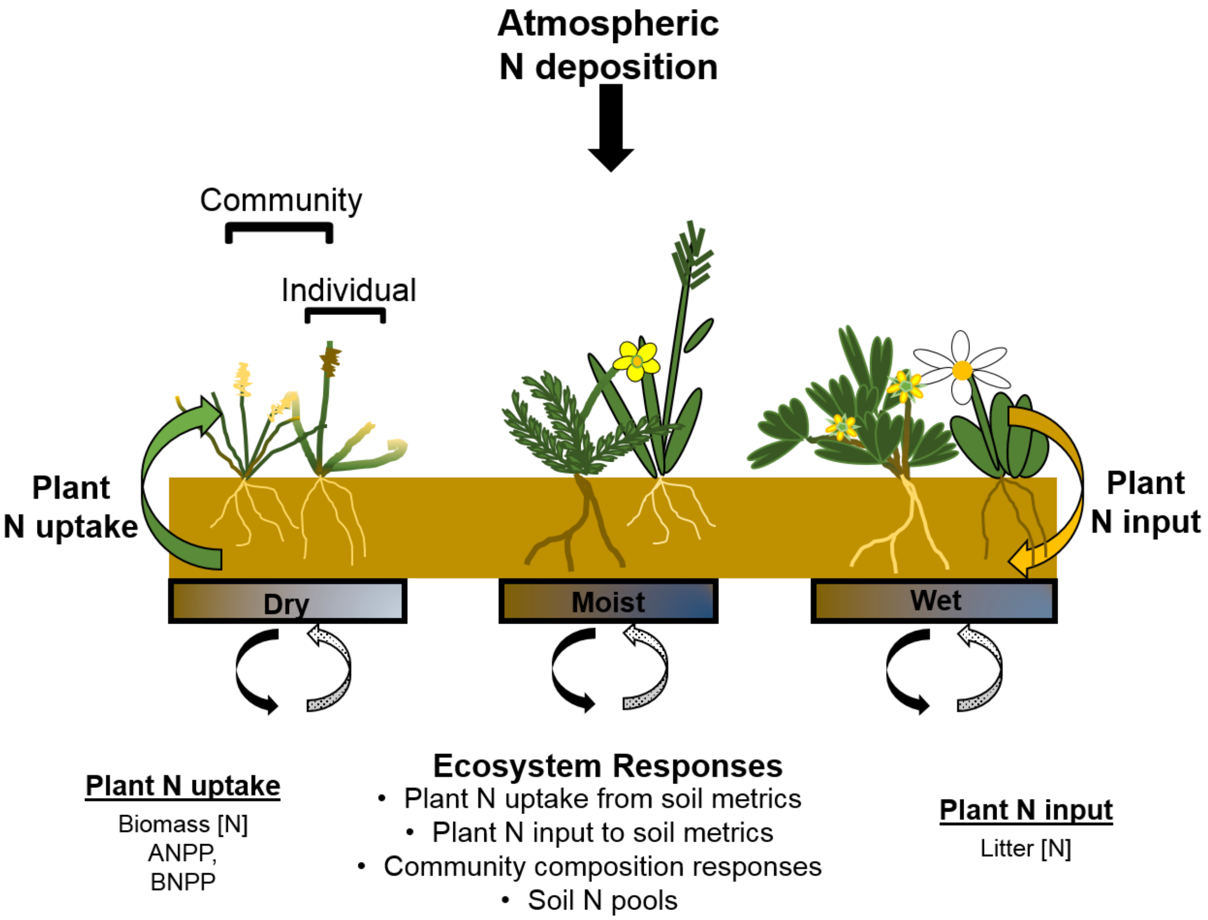

Figure 1.1 Conceptual framework linking plant and soil feedbacks of N cycling and how these feedbacks contribute to ecosystem responses under N deposition among three Rocky Mountain alpine communities (dry, moist, and wet communities; relative resource availability indicated by color saturation). Under ambient conditions, there are a variety of ways for plants to influence N cycling and these are indicated generally by the plant N uptake (green arrow) and plant N input (brown arrow) arrows from and into soils. The influence of plants is linked mechanistically to individual plants or species however net plant contributions to ecosystem processes occur at the scale of the entire plant community. Under conditions of elevated N inputs there are a number of plant responses that provide potential indicators of shifts in plant-ecosystem feedbacks (Ecosystem responses; black arrows) that I measured in the course of my dissertation.

changes (annual plants) to variation over multiple years (trees), and the longevity of these within-plant N pools is an important feedback influencing ecosystem responses to atmospheric N deposition among multiple terrestrial ecosystems (Currie et al. 1999, Lovett et al. 2000, Perakis and Hedin 2001, Friedrich et al. 2011).

Eventual plant loss of N in litter (**Figure 1.1**; Plant input of N to soil, brown arrow), is another process linking plant control of the N cycle with ecosystem functional responses to N additions (Hobbie 1992, Steltzer and Bowman 1998, Chapman et al. 2006, Yelenik and Levine 2011, Vivanco and Austin 2011). This process cycles aboveground plant carbon and N back into soil pools, and can have lasting effects on the development and structure of soil. Generally, low quality litter sources contribute to slower microbial processing and subsequent cycling of N, while high quality litter decomposes more rapidly and promote faster N cycling. Variation among plant species litter N is additionally affected by resorption, which allows plants with conservative N-use strategies to resorb nutrients from aboveground biomass into storage organs before senescence, therefore producing low quality litter (Korner 2003). Alternatively, plants without such strategies may produce high quality litter containing high levels of nutrients (Hobbie 1992, Chapman et al. 2006). Many plants also increase the quality of litter under conditions of increased availability of N (Chapman et al. 2006, Manning et al. 2008). Therefore, plant species that are able to rapidly take up newly available N, and incorporate that N into tissue and ultimately litter, promote a fast-track model of N cycling (Bowman and Steltzer 1998). In comparison, the litter chemistry of some plants enhances microbial immobilization of N resulting in at least a short-term reduction of N availability, thereby promoting slower rates of N cycling (Bowman et al. 2004, Meier and Bowman 2008). Litter quality is known to play an important role in rates of decomposition and N stabilization in both the litter layer and in soil organic matter, making it critical to build understanding of this feedback in order to determine plant contributions to ecosystem response with N deposition (Neff et al. 2002, Fahey et al. 2011, Hobbie 2015, Riggs et al. 2015). Changes in plant composition of communities with N addition, for example, therefore may alter the composition and abundance of litter chemical compounds, which ultimately mediate ecosystem recycling of C and N.

All of these individual plant processes contribute to N cycling within an ecosystem however it is the net contribution of the plant community, combined with microclimate soil characteristics, that ultimately determines subsequent ecosystem responses to N deposition (**Figure 1.1**; Community scale). One community-scale feedback that has received substantial experimental and observational attention is the relationship between plant community structure, usually in the form of species diversity, and ecosystem processes (Chapin et al. 2000, Suding et al. 2006, Loreau et al. 2008, Hooper et al. 2012, Cowles et al. 2016). In general, studies have found that greater diversity contributes to greater ecological stability in the face of disturbance, either through increased resistance or resilience (Folke et al. 2004, de Mazancourt et al. 2013, Loreau and de Mazancourt 2013, Mori et al. 2013). These studies have emphasized the importance of functional diversity, response diversity, and genetic diversity (Hooper 1997, Díaz and Cabido 2001, Elmqvist et al. 2003, Hooper et al. 2005, Reusch et al. 2005, Mori et al. 2013, Isbell et al. 2015), but have primarily focused on variation in these components within a single community type. As a consequence, studies are often limited to a single set of potential species interactions, which may or may not be maintained during diversity manipulations when creating artificial communities, possibly leading to underestimates of community influence on ecosystem responses (Tilman et al. 2012, Farrer et al. 2015). Theoretical work suggests that a primary mechanism of community-driven stabilization of ecosystems in response to disturbance is associated with the strength of interspecific interactions (as competition), such that weaker competition within a community promotes increased stabilization of the ecosystem (Loreau and de Mazancourt 2013). The effect of different interactions among species can be examined by adopting a comparative framework for different communities in determining the relative importance of biotic feedbacks to ecological thresholds of ecosystem change (Clark et al. 2007). Comparisons are particularly useful among communities with similar soil development and climate (Amundson and Jenny 1997), to partition feedbacks within the larger ecosystem in response to a common environmental driver (Field et al. 2014).

Alpine ecosystems and N deposition

Nitrogen deposition levels, and potential environmental impacts on ecosystem processes, are greater in regions with increasing populations of people and agricultural development such as along the Front Range in the southern Rocky Mountains (Baron et al. 2000, Nanus et al. 2003, Benedict et al. 2013b). Elevational increases in N deposition, combined with increased rates of deposition in association with human proximity, have made the alpine of the southern Rocky Mountains an important location for studying ecosystem responses and processes under elevated N deposition.

Alpine communities tend to exhibit non-linear responses to N additions, with threshold levels of nutrient addition necessary to induce change (Bowman et al. 2006, 2012). For the Rocky Mountain alpine, evidence from empirical and observational research shows that even levels of N deposition at or below 4 kg N ha^{-1} yr^{-1} significantly alter species composition (Baron 2006, Bowman et al. 2006, 2012). Alpine dry meadow communities show a significant increase in abundance for two species (*Carex rupestris* and *Trisetum spicatum*) in response to increased levels of N addition (Bowman et al. 2006, 2012). Additionally, abundance or biomass has declined for some species, including one of the co-dominant moist meadow species (*Geum rossi*) and a dominant dry meadow species (*Kobresia myosuroides*) (Bowman et al. 1993, Suding et al. 2008, Dean et al. 2014, Gasarch and Seastedt 2015). These tradeoffs in abundance show that the preferential growth of just a few species can alter biotic interactions and produce changes in plant composition (Miller and Bowman 2003, Bobbink et al. 2010). Changes in abundance of a single species is frequently linked to changes in productivity at the ecosystem level, further driving competitive interactions that reinforce initial changes in species abundance and diversity (Bowman et al. 1993, Theodose and Bowman 1997, Suding et al. 2005).

At present, most studies examining the effects of N deposition in the alpine have focused on dry meadows, with less attention to moist meadows and wet meadows. No studies have directly compared how these three communities respond to N deposition, though some evidence suggests each community may respond differently to nutrient additions (Bowman 1994, Bowman et al. 1995, Gasarch and Seastedt 2015). Additionally, these studies have been primarily located in alpine areas already receiving elevated levels of ambient N deposition where the communities and associated soils have accumulated N over the past four decades. Finally, N addition studies in the alpine historically included concentrations much higher than projected ambient deposition levels, making it difficult to accurately calculate ecosystem responses and critical loads for low levels of deposition. Despite these limitations, ambient levels of N deposition in many federally protected lands in Colorado are approaching, or have exceeded, the current estimated critical loads for changes in species composition (Bowman et al. 2006, 2012). Consequently, there is increased interest in understanding community scale differences in responses and contributions to ecosystem change with N deposition, both at a landscape scale as well as among communities experiencing similar conditions (Nanus et al. 2003, Porter and Johnson 2007, Beem et al. 2010).

Overview of research

Responses to N deposition can include changes in plant biomass, species abundance and community composition, as well as a number of ecosystem functional responses such as changes in concentrations of soil solution nitrate (NO_3^-), changes in soil pH, and changes in soil cation exchange capacity (CEC). Plants and soils can interact to produce feedbacks that may alter the ecological N thresholds associated with N deposition. These interactions can either provide stabilizing feedbacks that minimize changes in ecosystem function in the face of environmental disturbance, or amplifying feedbacks that intensify the effects of environmental change and may promote irreversible alterations (Chapin et al. 2010). While we know that plants influence ecosystem structure and function, there are many research gaps associated with plant to soil ecosystem feedbacks in response to environmental change (Chapin et al. 1997). Due to increasing levels of N deposition, and substantial research on plant interactions in alpine meadow communities, the southern Rocky Mountain alpine is a unique system to examine community scale differences in ecosystem responses that are associated with underlying differences in plant-soil feedbacks.

The research presented herein focuses on the ecological effects of ambient nitrogen deposition in one alpine plant community throughout the Southern Rocky Mountains (Chapter 2), differences in responses of plant and soil indicators of ecological change among three alpine plant communities under elevated N deposition (Chapter 3), and determining the importance of different pools of N in stabilizing reactive N among three alpine plant communities responding to elevated N deposition (Chapter 4).

CHAPTER 2
Alpine moist meadow response to a regional gradient of nitrogen deposition in the Rocky Mountains

Abstract

Human alteration of the nitrogen (N) cycle has resulted in an increase in availability of biologically active N. Alpine ecosystems are particularly susceptible to increased inputs of N due to shallow soils and a short growing season, and they are exposed to higher rates of N deposition than surrounding lower elevation areas making them a good indicator system for ecosystem responses to N deposition. I examined variation among sites receiving a range of ambient N deposition in alpine moist meadow communities (3.3 to 6.2 kg N ha^{-1} yr^{-1}), and determined whether differences in plant and ecosystem metrics were correlated with N deposition. I found that a number of ecosystem metrics were correlated with ambient N deposition, including the tissue N concentration of two dominant plant species at peak biomass and the abundance of a nitrophilic dominant grass species. These species are known to have an influence on N processing in soil, and are therefore important potential plant-soil feedback mechanisms facilitating future ecosystem responses to environmental changes. Additionally, I found that there were significant differences in species composition among sites receiving different levels of N deposition, although there was only a weak negative correlation between diversity and N deposition. I found a significant positive correlation between nitrate concentration in soil solution and N deposition during two years of sampling, while all other soil metrics showed no changes with N deposition. These findings suggest that moist meadows in the southern Rocky Mountain alpine are being affected by N deposition with minimal soil biogeochemical changes but substantial biotic impacts.

Introduction

Human alteration of the nitrogen (N) cycle has resulted in a substantial change in the availability of reactive N in many regions, and is likely to continue so into the future (Vitousek et al. 1997, Dentener et al. 2006). Reactive N moves from emission sources, such as areas of high fossil fuel combustion or animal feed lots, to natural areas where that N is deposited as either wet or dry deposition (Fenn et al. 2003b). Nitrogen deposition levels, and potential environmental impacts, usually increase in regions near expanding human populations and/or changes in land use that create reactive N. Consequently, N deposition has been identified as one of the global change factors most highly affected by humans (Baron et al. 2000, Matson et al. 2002, Rockström et al. 2009b, Steffen et al. 2015). Nitrogen deposition has been previously associated with numerous ecosystem responses, including eutrophication, nitrate leaching into ground water, nitrous oxide production, loss of plant diversity, and the acidification of soils and surface waters (Pardo et al. 2011).

Research measuring ecosystem responses to N deposition has primarily utilized experimental fertilization studies or gradients of N deposition within a single community type (Aber et al. 1989, Zak et al. 1990, Isbell et al. 2015, Stevens et al. 2015). Fertilization studies often use levels of N additions, far greater than projected levels due to increased emissions, to initiate a rapid measurable ecosystem response. As a consequence, many studies have not been designed to detect the gradual changes of individual plants, shifts in populations of key species or changes in community structure that could occur with accumulation of N due to deposition. Gradient studies investigating sites with a range of N deposition inputs can highlight ecosystem responses to gradual increases in N deposition using a space-for-time proxy. This method has successfully been applied to analyze ecosystem responses in forest and grassland ecosystems in New England (Aber et al. 1998, 2003), the southern Appalachians (Boggs et al. 2005), and

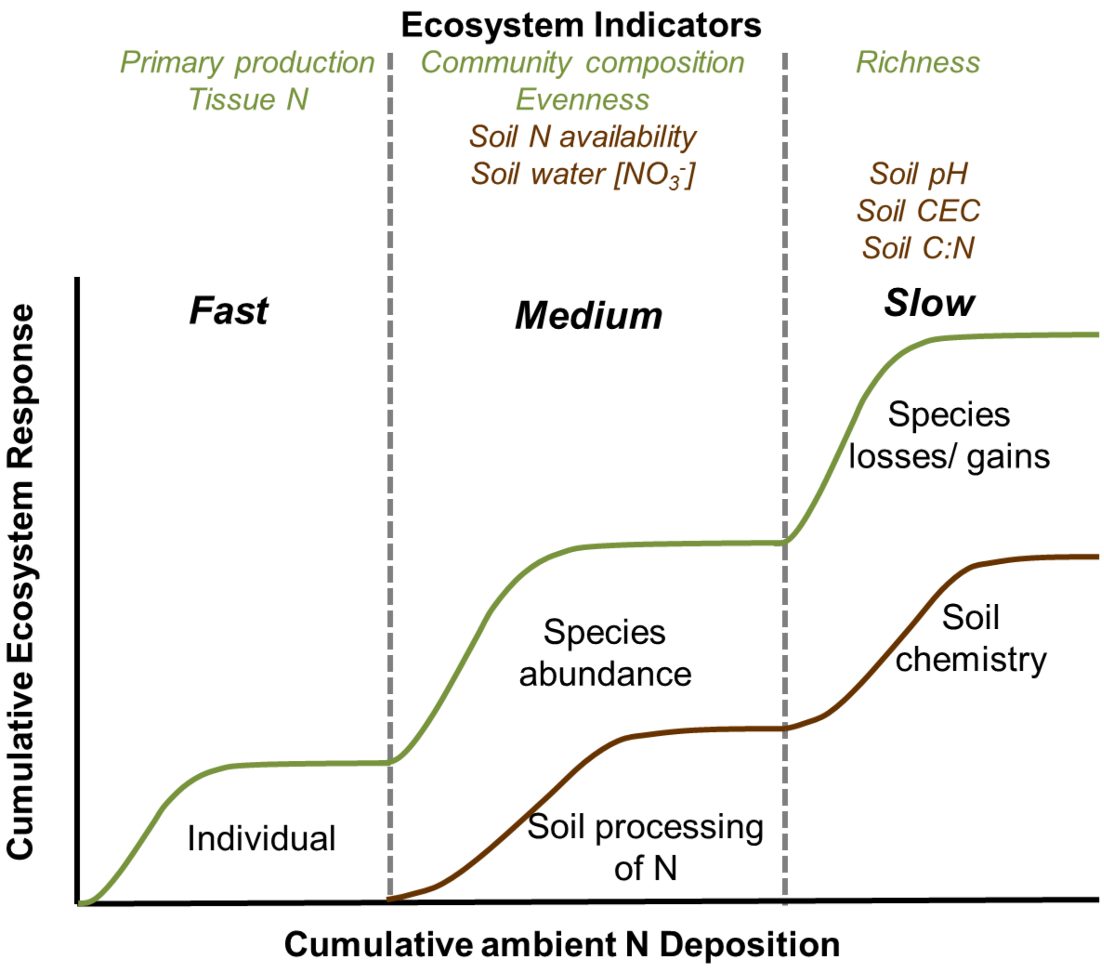

Figure 2.2 Ecosystem responses (indicators of increasing chronic N deposition) that are expected to occur within a hierarchical framework for plant based components (green curve) and soil components (brown curve). This framework is based on previous predictions of plant and soil responses to chronic resource changes (Aber et al. 1998, Smith et al. 2009), and allows us to qualitatively examine which indicators in an ecosystem have a relationship with N deposition.

across Europe (Stevens et al. 2004, 2006, 2010a, Duprè et al. 2010, Maskell et al. 2010, Gaudnik et al. 2011). Gradient studies involve observational data collected across sites, and they can encompass lower levels of N inputs than the lowest treatment level of most fertilization studies. This design therefore allows an examination of the consequences of N deposition among ecosystem responses (**Fig. 2.1**) otherwise difficult to monitor in fertilization studies (Smith et al. 2009).

Ecosystems respond to N deposition through integrated changes where the occurrence of one response is dependent upon previous responses, and individual responses can be used as potential indicators that are either plant or soil focused (**Fig. 2.1**; Aber *et al.*, 1998; Smith *et al.*, 2009). My proposed framework builds on conceptual models of individual plant and soil metrics to increased N (Aber et al. 1998) by placing individual changes in the context of other processes being altered, similar to the response framework of Smith *et al.* (2009) and the relationship of changes in processes with measureable response metrics used by Lovett & Goodale (2011). Changes in plant and soil ecological indicators within my framework are associated with total accumulation of N as an integration of the duration of exposure to N deposition (Smith et al. 2009) and the magnitude of N additions. Under my hypothesized framework, the cumulative magnitudes of responses are associated with integrated changes in ecosystem indicators as the total N inputs increase. Based on predicted dependencies between individual plant and soil indicators, they can be classified into fast, medium, and slow stage ecosystem responses. The initial responses, or “fast indicators” are predicted to initiate under relatively low increases in N deposition conditions (≤ 2 times ambient conditions) (Bowman et al. 2006, Bobbink et al. 2010, Phoenix et al. 2012, Simkin et al. 2016). On the other hand, responses that occur under higher levels of N accumulation, or “slow ecosystem responses” associated with advanced stages of N saturation (Aber et al. 1998), are generally less reversible than changes in fast or medium indicators (Clark and Tilman 2010).

Fast indicators of plant responses to elevated N deposition may include increased growth of individuals (Hobbie, 1992; **Fig. 2.1**) or increased luxury uptake and storage of N (Hobbie 1992) as some ecosystems may have physiological limitations associated with growth (Billings 1974, Korner 1989). Proposed medium stage indicators include changes in the abundance of species within a community once growth responses alter species interactions (**Fig. 2.1**). Changes in abundance as N deposition increases is predicted to predominantly affect species adapted for either low N availability or high N availability (nitrophilic) where the former tend to decline as nitrophilic species proliferate under chronic N deposition (Bassin et al. 2007). The preferential growth of nitrophilic species in ecosystems impacted by N deposition may also alter competitive interactions within the plant community and can lead to a shift in plant community composition and either decreases or increases in diversity (Bowman et al. 2006, Bobbink et al. 2010, Simkin et al. 2016). Medium stage indicators for soils associated with increased N deposition include increased rates of N cycling and mineralization (Aber et al. 1998, Baron et al. 2000), potentially leading to increased concentrations of soil pore water nitrate ($[NO_3^-]$) if biotic uptake doesn't keep pace with production (Vitousek *et al.*, 1997; Fenn *et al.*, 1998, 2003b; **Fig. 2.1**). As N deposition increases, slow stage plant responses such as loss of species may occur (reviewed in Bobbink *et al.*, 2010), along with soil responses dependent on increased nitrate concentrations in soils that contribute to leaching of soil base cations and promote soil acidification (Driscoll et al. 2003, Bowman et al. 2008).

Alpine ecosystems are particularly susceptible to elevated N deposition as there is limited potential for storage or stabilization of reactive N by poorly weathered soils and the short growing season that limits biotic uptake (Billings 1974). Generally, N is considered a nutrient limiting for plant growth in the alpine ecosystems (Bowman et al. 1993, 1995), however many of the native plants exhibit slow growth rates, lowering resource requirements (Forbis and Diggle 2001, Korner 2003). As a consequence, the addition of anthropogenic N has the potential to disrupt interactions between soil and plants that developed under low ambient levels of N deposition. Parts of the Rocky Mountains experience enhanced N deposition associated with greater levels of precipitation at high elevations and increased proximity to anthropogenic N sources (Williams and Tonnessen 2000, Nanus et al. 2003) providing a gradient of N deposition suitable for evaluating potential ecological responses (Nanus et al. 2003, Simkin et al. 2016).

In this study I examined a key question: How well do ecological metrics correlate with N deposition across an ambient gradient of deposition in alpine moist meadow communities of the southern Rocky Mountains? I established five research sites along this gradient, including three sites on the eastern slope of the Front Range in CO with relatively high levels of ambient N deposition, and two sites with lower ambient deposition. I predicted that I would find stronger correlations between fast (ANPP; plant tissue N concentrations) and medium indicators (diversity, community composition, and soil N availability) with ambient N deposition, and weak or no correlations for slow ecological indicators (plant species richness, soil pH, cation exchange capacity and soil carbon to N ratios; **Fig. 2.1**) based on the comparatively low levels of ambient N deposition in the region.

Methods

Site Description and Experimental Design

Sites were selected based on estimated annual N deposition levels obtained by combining data from the National Atmospheric Deposition Network for wet deposition and the Community Multi-scale Air Quality modeling system for dry deposition (**Fig. 2.2**; Simkin *et al.*, 2016). These modelled estimates were used to verify differences in N deposition among the sites. I installed three sites in 2012, including Niwot Ridge (Niwot; Colorado), Rocky Mountain National Park (Rocky; Colorado), and Shoshone National Forest (Shoshone; Wyoming), and the remaining two sites were added in 2013 including Fraser Experimental Forest (Fraser; Colorado) and Arapaho National Forest (Torreys; Colorado; **Table 2.1**). The research was performed in moist meadow alpine communities (*sensu* Walker *et al.*, 2001), characterized by the presence of two dominant plant species (*Geum rossi*- a forb and *Deschampsia caespitosa*- a grass). These two dominant species were selected for emphasis as they differ in their capacity to respond to N additions (Bowman and Steltzer 1998) where one is nitrophilic (*D. caespitosa*) and the other shows limited changes in growth associated with changes in N supply (*G. rossii*; Miller & Bowman, 2003). The sites ranged in elevation between 3198-3649 m. All sites had flat to < 15% slopes, with similar soil development (cryumbrepts derived from granitoid parent material; Seastedt, 2001), except for the Shoshone site, which had igneous volcanic derived bedrock in the Absaroka Volcanic Supergroup (Love and Christiansen 1983). Additionally, all sites on the eastern side of the Continental Divide had an eastern aspect, while Fraser had a western aspect. Samples were collected during the growing seasons of 2012- 2014. At each site I established three replicate blocks in individual moist meadow communities located within 0.5km of each other, to account for microsite differences that could influence variation in my measurements. Replicate plots were then established in each block, with the number of samples collected from plots, nested within blocks, associated with different metrics as described below.

Site measurements of N deposition, climate and weather, and soil texture

In addition to the modelled estimates of annual nitrogen deposition at each site, I measured growing season bulk deposition of N (June 1- August 30) using ion-exchange resin columns installed in the field (Fenn et al. 2009). These measurements of summer N deposition were used for examining correlations with ecosystem metrics of change, although they only represent a portion of the total annual deposition at a given site (Benedict et al. 2013a, Clow et al. 2015). Columns were attached to 21 cm diameter funnels for bulk 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 I charged using 0.5 M NaCl prior to deployment. I calculated absorption and extraction efficiencies for each batch of resin (Fenn et al. 2009). During installation I attached each collector on top of 0.60 m PVC pipes that were inserted into the ground to a 0.20 m depth for stability. For 2013 and 2014 I installed five collectors adjacent to the moist meadow study area. After retrieving from the field, resin in the collectors was processed as described below for resin bags. Wet deposition of N is monitored at Niwot Ridge and Rocky Mountain National Park as part of the National Atmospheric Deposition Program (NADP; http://nadp.sws.uiuc.edu/). Precipitation chemistry is monitored for the Fraser Experimental Forest (Fraser) using methodology comparable to the NADP.

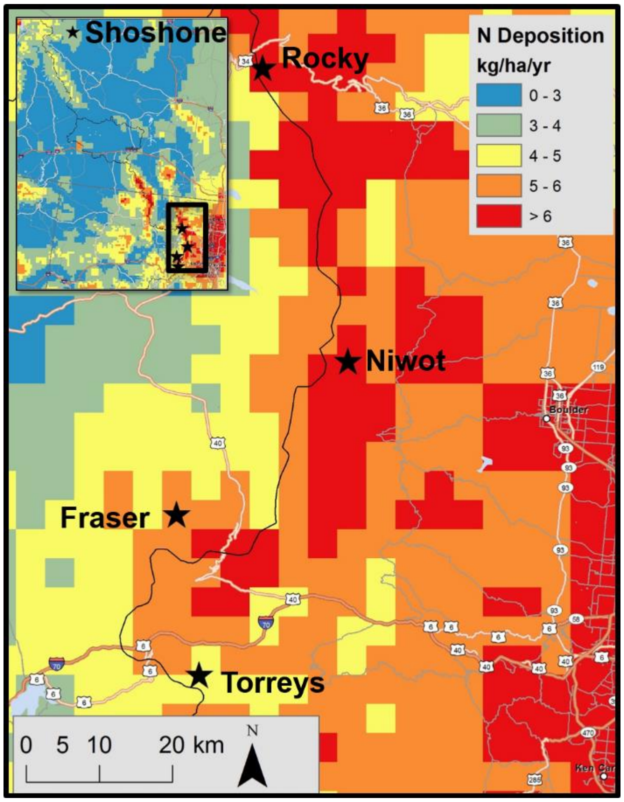

Figure 2.2 Regional map of ambient nitrogen deposition, based on modelled wet and dry deposition for the Rocky Mountains, with study sites indicated.

I compiled a long term mean annual temperature and precipitation record (1981-2011) for each site, based on meteorological stations present within 10km of my sampling areas, as well as weather data associated with my specific study years (2012-2014), to examine potential differences in weather factors among the sites. Data for Torreys were measured at the Grizzly Peak SnoTel site, and weather data for Niwot were collected at the D1 meteorological station run by the University of Colorado’s Mountain Research Station. Long term climate data for Fraser were measured at the

Fraser Experimental Forest Headquarters (US Forest Service, Kelly Elder, personal communication), while weather data associated with the study years was from the Fool Creek SnoTel site within the Experimental Forest. Rocky precipitation and temperature data were collected at the Lake Irene SnoTel site, and Shoshone data at the Kirwin SnoTel site (all SnoTel data from http://www.wcc.nrcs.usda.gov/snow/). Temperature data showed less variation among sites than the precipitation data (Table 1), and so I focused on precipitation in my analyses to account for site differences explaining variation in my response metrics.

Table 2.1. Site characteristic summary

Site	**Latitude**	**Longitude**	**Elevation (m)**	**Precipitation (mm)**†	**Annual T_{max}**†	**Annual T_{min}**†	**% Clay**	**N deposition (kg N ha^{-1} yr^{-1})**‡
Fraser	39.84839	-105.85656	3600	860	4.96	-7.51	17	4.8
Niwot	40.05655	-105.60578	3649	1208	4.42	-8.04	31	6.2
Rocky	40.40343	-105.79245	3600	919	4.36	-8.01	28	5.9
Shoshone	43.93020	-109.29321	3198	668	3.89	-7.33	23	3.3
Torreys	39.65538	-105.79324	3637	826	4.99	-7.2	28	4.4

†Mean annual precipitation, and annual maximum and minimum temperature (1981-2011) measured at meteorological stations within 10 km from sampling locations

‡Mean annual deposition of wet + dry nitrogen as calculated by (Simkin *et al.*, 2016)

I collected 15 cm deep soil samples (A horizon) within each block (n = 3 for each site) to characterize potential differences in soil textures among the different site locations. Texture was analyzed using a hydrometer for particle size analysis (Soil, Water, and Plant Testing Laboratory; Colorado State University). Potential site differences in bedrock chemistry were evaluated using representative rock samples from each site using Whole Rock Analysis with a four-acid digestion (Briggs and Meier 1999) for composition of elements in each sample (IPC-OES analysis, Laboratory for Environmental and Geological Studies, University of Colorado Boulder).

Measuring ecological metrics of ecosystem response to N deposition

At each site I measured ecosystem metrics that were predicted to respond to variation in N deposition based on my proposed conceptual framework (**Fig. 2.1**). Fast stage plant metrics included aboveground net primary production (ANPP) of the two dominant species as well as total vascular plant ANPP and concentrations of N in foliar tissues and litter. Medium stage metrics for plant components included cover of the dominant species, plant community diversity, and community composition based on total species cover. Slow stage plant metrics included plant species richness. Soil metrics at the medium stage included pore water concentrations of NO_3^- and resin extractable soil N (resin bag), and the slow stage soil metrics included pH, cation exchange capacity (CEC), and carbon (C) to N ratios and concentrations in bulk soil.

Plant Measurements

I clipped all aboveground vascular biomass at peak season (end of July), to estimate aboveground vascular net primary production (ANPP), within one 20 cm by 20 cm subplot for at least 10 plots per block at all five sites in 2014 (n = 155 samples total). These biomass harvests were sorted in the laboratory to separate current year's plant growth from litter. New growth was further divided into ANPP of each moist meadow co-dominant plant species (*D. caespitosa* and *G. rossii*). All samples were then oven dried at 60 °C for 48 hours before weighing on a Mettler AE 100 balance.

I collected aboveground tissues from *D. caespitosa* and *G. rossii* individuals from ~10 plots in each block in 2013 both at peak biomass (late July; n = 125) and again following senescence in late August (n = 123 samples total). All plant tissues were oven dried at 60 °C as above, and then homogenized and ground to a fine powder using liquid N and a mortar and pestle. These samples were then analyzed for N concentration using a CHN autoanalyzer (Flash 1112 Series).

I measured the cover of vascular plants species in 2013 (all sites) using a point-intercept method with a 10 x 10 grid of 100 points in each plot (vegetation plots were 1 m x 1 m). I measured species cover in twenty plots in each of three blocks at Niwot, Rocky, and Torreys (n = 60 at each site), while at Fraser I measured a total of twenty plots (1 block; n = 20), and at Shoshone I measured 10 plots in each block (3 blocks; n = 30; species area curves saturated around 5 plots- data not shown). Plant species that were present within a plot but not recorded at one of the points were assigned a projected cover value of 0.5%. Due to a possible leaf area index higher than 1, total projected vegetation cover within a plot could be greater than 100%. To calculate the relative abundance of dominant plant species, I determined the proportion of total cover associated with each species. Species nomenclature followed USDA Plants database (http://plants.usda.gov). Species diversity was calculated for each plot following the Shannon Diversity Index (H'), and richness as the number of species within a plot.

Soil Measurements

Growing season plant available dissolved inorganic N (DIN) was estimated using ion exchange resin bags, constructed using plastic cylinders (3.8 cm^2, 1.6 cm height, 6.1 cm^3 volume), covered with nylon mesh, containing mixed bed exchange resin beads (IONAC NM-60 H^+/OH^- Form; J.T. Baker). All resin bags were charged using 0.5 M NaCl prior to deployment. The resin bags were inserted to a depth of 10 cm, horizontally within the rooting zone to capture vertical movement of soil N pools, in five plots in all three blocks at a site for both 2013 and 2014 (n = 15 per site per year). Resin bags remained in the field for the duration of the growing season (52-101 days range among sites and years), and upon removal I stored samples at 10 °C until extraction with 2M KCl. Extracts were analyzed for NO_3^- and NO_2^- using a Lachat QuikChem 8000 Specto- Flow Injection Module (Bowman et al., 2012) and for NH_4^+ using a BioTek Synergy 2 Multi-Detection Microplate Reader. I determined adsorption and extraction efficiencies for each batch of resin and applied a correction factor as necessary to the final extraction values for field samples, as well as determining any potential transport contamination of the resins using field blanks (Fenn *et al.*, 2009). I acquired soil pore water for measuring the concentration of dissolved inorganic N using vacutainers attached to microlysimeters installed in five plots in all three blocks at a site for both 2013 and 2014 (n = 15 per site; Rhizon soil moisture samplers, Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands). I sampled soil pore water as a point measurement at the start of the growing season, as close to snow melt as I could access the sites. Soil water samples were analyzed for concentrations of NO_3^- as described above, however I did not detect dissolved NH_4^+ as concentrations were below detection limits. Measurements of soil available N from pore water concentrations of NO_3^- and resin bags integrate over different time periods, and therefore capture different N cycling processes that can vary throughout the growing season, such as changes in microbial biomass (Lipson et al. 1999).

I collected bulk soil samples from 5-20 plots in each block of each site (total n = 135), to examine variation within and among sites in soil pH, CEC, and carbon (C): N ratios. Soil samples included the A horizon, to a depth of 15 cm, with a total volume of around 1500 cm^3. I collected soils at Niwot, Rocky, and Shoshone in 2012, soils at Torreys and one block at Fraser in 2013, and the remaining Fraser samples in 2014. All soils were air dried in the laboratory and then sieved to a 2 mm fraction before subsampling for separate analyses. To measure soil pH, I used a Beckman 340 pH probe on a 2:1 ratio of nanopure water to soil paste determined after 30 seconds of shaking, using soils from five plots in each block at each site (n = 75 samples total). I extracted another subset of soil with 0.1 M $BaCl_2$, and analyzed the extract for base cations and Al^{3+}, Fe^{3+}, and Mn^{2+} using an ARL Inductively Coupled Plasma Atomic Emission Specrophotometer (ICP-AES; University of Colorado Boulder), for the same five plots in each block at each site (n = 75 samples total). A third subsample was oven dried at 105 °C, and homogenized using a ball mill (Cianflone Scientific Instruments Corporation) before analyzing for C and N concentration as described above. Soil % C and N was determined from subsamples of soil from 20 plots in each block at Niwot, 15 plots in each block at Shoshone (one block has 20), 10 plots in each block at Rocky and one block at Torreys, 5 plots in two blocks at Torreys and 5 plots per block at Fraser.

Statistical analyses

To examine how my field measurements of N deposition corresponded with other short-term and long-term estimates of N deposition, I analyzed the correlation between my on-site measurements of N deposition and the local monitoring programs' measurements, as well as between my on-site measurements and estimates of long-term N deposition derived from interpolated wet deposition and modelled dry deposition (**Fig. 2.2**; Simkin *et al.*, 2016). I used a linear mixed model in the R nlme package to determine potential differences in field measured N deposition among sites, with block as a random effect and site as a fixed effect (Pinheiro et al. 2014), followed by an analysis of variance (ANOVA) test among sites. All potential differences among sites were then examined using post hoc Tukey comparisons in the R multcomp package (Hothorn et al. 2008). All analyses in R used version 3.2.3 (R Core Team 2015).

To examine the correlations between ecosystem metrics and levels of N deposition as measured in the field, I used mixed effects models in the R nlme package (Pinheiro et al. 2014). All models for data collected in a single year were fit with the same random effects structure, including block nested within sites and the block level clay content of soil as a proxy for potential differences in soil moisture (Cosby et al. 1984). Site was included as a random effect to account for potential differences in latitude, bedrock, and climate, and to allow the examination of the contributions of N deposition in influencing my measured responses specifically. For the fixed effect I used the average N deposition measured at each site in 2013 and 2014. For metrics where data were collected in both 2013 and 2014 I used N deposition from that site in the year the data were collected as the fixed effect, and included year as a random effect to account for repeated measures (resin extractable soil N and pore water [NO_3^-]). I determined the total variation described by these models using the R MuMIn package (Barton 2015), and I report relative fit based on calculations of marginal R^2, which includes variation described by fixed effects only (N deposition), and conditional R^2, which includes variation described by both random and fixed effects (N deposition, as well as site/block and soil clay variation among blocks), to calculate the total error described by the full model (Nakagawa and Schielzeth 2013, Johnson 2014). I also report effect size associated with fixed effects as a measure of the ecological relevance of the relationship between the predictor and response and p values associated with the slope of each model. Data for ecological metrics were transformed as necessary to meet assumptions of normality and constant variance, which included a square root transformation for dominant plant species ANPP and proportional relative abundance, a fourth root transformation for resin extractable soil N, and log transformation for soil water concentration of NO_3^- and soil pH.

For multivariate data comparisons among sites, I used a Multiple Response Permutation Procedure hypothesis test to compare plant species composition, based upon a Bray-Curtis dissimilarity matrix (PC Ord 5.3.1; MJM Software), and I report the total variation associated with each axis as well as the effect size of including site as a grouping factor (*A*: chance corrected within-group agreement; McCune & Grace, 2002). I used a non-metric multi-dimensional scaling ordination, based on Sorensen dissimilarity to visualize potential differences between sites, and examined the Pearson correlation of environmental data with each ordination axis, including plot scale latitude, longitude, and elevation, and site scale information for long term average N deposition, 2013 growing season N deposition, long term average precipitation, and water year 2013 precipitation totals as potential environmental correlates (**Table 2.1**).

Differences in ecosystem metrics among my five sites could be driven by a variety of factors, including (but not limited to) N deposition, climate, soil texture, and bedrock chemistry. To determine whether the N deposition models could be improved by including precipitation, as these two processes are necessarily confounded in the field, I applied a model selection of fixed effects including N deposition and precipitation using the R MuMIn package (Barton 2015). The fixed effects for N deposition included the same values used for the models described above, while precipitation data were long-term annual estimates of precipitation (described above). Best fit models were determined using corrected Aikike Information Criteria (AICc), calculated using restricted maximum likelihood. I report models with the lowest AICc associated with each ecosystem indicator (± 2 AIC), as well as relative fit based on calculations of marginal R^2 and conditional R^2 as described above. Site bedrock chemistry was examined using principle components analysis (PCA) to visualize potential differences in element components among selected sites (PC Ord 5.3.1; MJM Software). Based upon these results, I re-ran the mixed effects models associated with soil metrics derived from bedrock components (soil pH and CEC) to exclude sites with dissimilar bedrock types due to the direct influence bedrock composition exerts on soil buffering capacity.

Results

Site measurements

Growing season N deposition rates measured from ion exchange resin columns were different among the sites ($F_{4,40}$= 11.17, $p < 0.001$; **Fig. 2.3**). Niwot received the most summer N deposition, followed by Torreys and Rocky, Fraser, with the lowest at Shoshone. Deposition during 2013 was greater than 2014 for all sites ($F_{1,40}$= 51.08, $p < 0.001$), and there was a marginally significant interaction between site and year ($F_{4,40}$= 2.50, $p = 0.058$), primarily driven by the level of inter-annual variability among sites. My measurements for growing season bulk N deposition between 2013 and 2014 were highly correlated with wet deposition measurements (National Atmospheric Deposition Program, Fraser Experimental Forest) during the same sampling interval ($R^2 = 0.74$, **SFig. 2.1**). Model predicted values for annual N deposition at each location were also correlated with my growing season measurements from the summers of 2013-2014 ($R^2 = 0.32$), despite the differences in duration and methodology between the two metrics of deposition.

The relationship between soil cations and precursor elemental bedrock oxides at each site varied with some common oxides underrepresented in cation concentrations (aluminum) and other more rare oxides overrepresented in the cation concentrations (calcium, magnesium) (**STable 2.1**). In general, all sites showed similar bedrock composition, with the exception of Shoshone having more Ca and Mg than other sites (**SFig.2. 2**/ **STable 2.1**). Clay content ranged from 17% at Fraser to 31% at Niwot (**Table 2.1**).

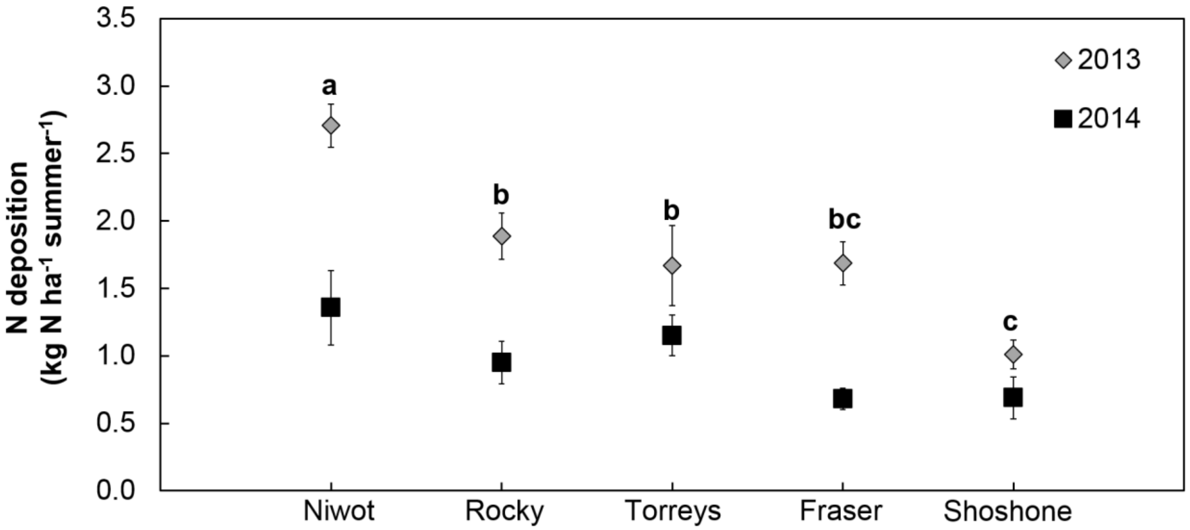

Figure 2.3 Mean field measured levels of nitrogen deposition from 2013-2014 using ion-exchange resin samplers installed in the field during summer months, error bars are ± 1 SE. Significant differences among sites are indicated with different letter designations (significant effect of site $p < 0.0001$ and year $p < 0.0001$).

Relationships of ecological metrics with N deposition

Plant based metrics of ecosystem change

I examined a total of thirteen different plant-based ecological metrics for potential correlations with N deposition as indicators of ecosystem change. These plant metrics included measurements hypothesized to follow fast, medium, and slow stages of change, and therefore were predicted to show differential strength in correlations with variation in ambient N deposition. My fast response stage metrics included ANPP for dominant species as well as the total vascular plant community and dominant plant species aboveground bulk tissue and litter N concentration; medium stage metrics included cover of the dominant species, community composition, and evenness of species within the community. My slow stage metric was species richness.

There was no observed relationship between N deposition and ANPP of both dominant plant species (**Table 2.2**), contrary to the expectation that these would be among the fastest responding plant metrics. However, there was a weak correlation between N deposition and total vascular ANPP as the variation described by the model was low (R^2-m = 0.06; **Table 2.2**). Including precipitation as a predictor for total ANPP and both dominant species' ANPP did not improve the amount variation described by fixed effects (**STable 2.2**).

Table 2.2 Ecosystem plant metrics model effect size and total variation associated with nitrogen deposition

Ecosystem indicator	Intercept	N Deposition (Measured)	R^2-m[†]	R^2-c[‡]	P Value
D. caespitosa[§] ANPP[¶] (g/m^2)	8.68 ± 2.57	0.16 ± 1.67	0.005	0.154	0.93
G. rossii[#] ANPP[¶] (g/m^2)	6.95 ± 2.56	-1.51 ± 1.79	0.006	0.375	0.41
ANPP (g/m^2)	125.6 ± 41.7	51.4 ± 29.1	0.055	0.252	0.10
D. caespitosa biomass N%	0.58 ± 0.24	1.13 ± 0.16	0.595	0.723	<0.001
G. rossii biomass N%	0.94 ± 0.22	0.83 ± 0.15	0.567	0.769	<0.001
D. caespitosa litter N%	1.05 ± 0.23	-0.01 ± 0.16	<0.001	0.616	0.97
G. rossii litter N%	1.27 ± 0.17	-0.16 ± 0.11	0.078	0.549	0.20
D. caespitosa Cover	0.03 ± 0.19	0.37 ± 0.13	0.310	0.830	0.02
G. rossii Cover	0.30 ± 0.09	0.02 ± 0.06	0.001	0.203	0.80
Diversity (H')	2.05 ± 0.26	-0.27 ± 0.17	0.069	0.471	0.15
Richness	17.1 ± 2.2	-2.17 ± 1.49	0.059	0.443	0.17

[†] Marginal R^2- variation associated with fixed effects only
[‡] Conditional R^2- variation associated with both fixed and random effects (soil clay content and block effect etc.)
[§] *Deschampsia caespitosa*, a dominant grass species present in alpine moist meadows
[¶] Aboveground net primary production (ANPP)
[#] *Geum rossii*, a dominant forb species present in alpine moist meadows

Medium scale plant metrics showed that both *D. caespitosa* and *G. rossii* tissue N concentration were positively correlated with increasing N deposition (**Fig. 2.4a, b**; **Table 2.2**). Adding precipitation as an alternative predictor of tissue N concentration was equivalent to N deposition, although models containing both predictors did not increase the total variation described (**STable 2.2**). On the other hand, litter N concentrations for either species were not correlated with N deposition (**Table 2.2**). Including both N deposition and annual precipitation as predictors of litter N concentration improved the overall model in describing the variation in

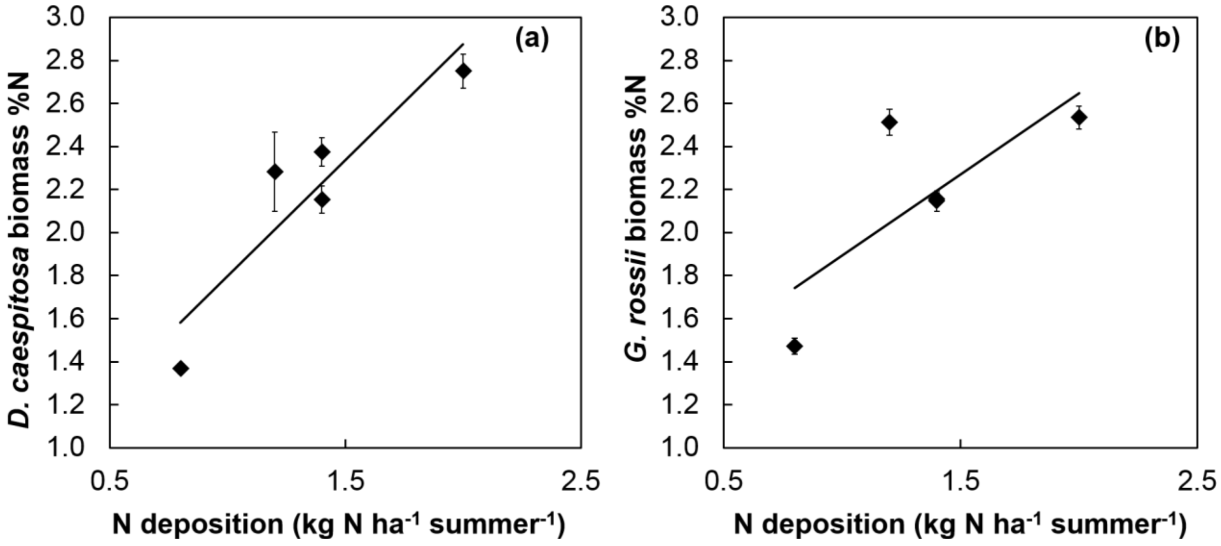

Figure 2.4 The relationship between growing season nitrogen deposition as measured in the field, and dominant plant species concentrations of nitrogen. Biomass concentration of nitrogen (N) in living tissue are shown for *Deschampsia caespitosa* (*D. caespitosa*) in panel **(a)**, and dominant plant *Geum rossii* (*G. rossii*) in panel **(b)**. Points are means ± 1 standard error, showing the correlation between measured summer N deposition and tissue N%.

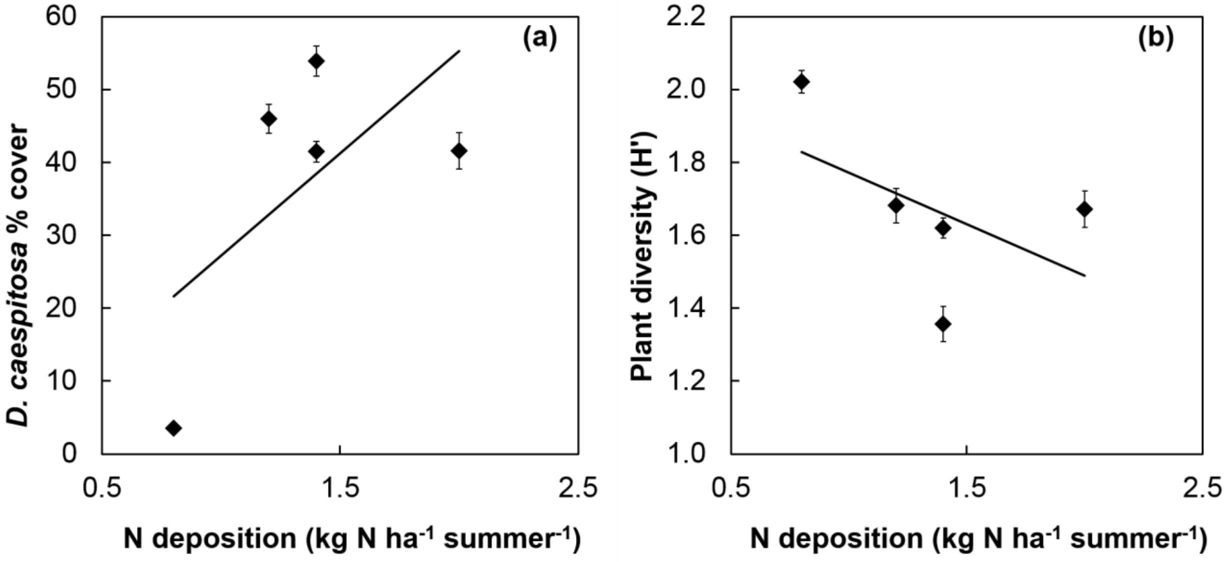

Figure 2.5 Relationship between field-measured growing season nitrogen deposition and plant medium stage ecological indicators including **(a)** proportional cover of *D. caespitosa* (2013), and **(b)** plant species diversity (2013). Points are means ± one standard error, with the best fit model showing the correlation between nitrogen deposition and each indicator.

D. caespitosa; however there was no similar improvement for *G. rossii* (STable 2). Dominant species cover showed differences in the strength of correlations to N deposition, with *D. caespitosa* cover being moderately correlated with growing season N deposition (R^2-m = 0.31; **Figure 2.5a; Table 2.2**). In contrast, cover of *G. rossii*, was not correlated with growing season

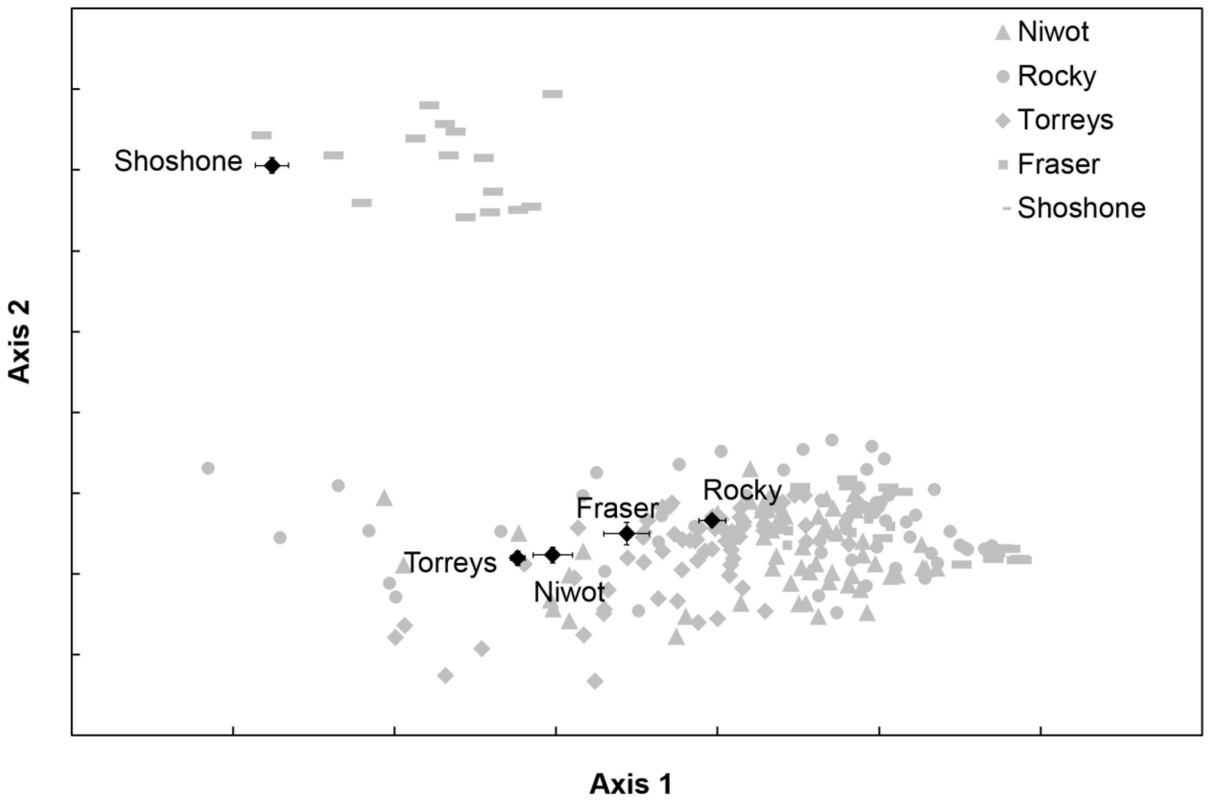

Figure 2.6 Non-metric multidimensional scaling ordination of plant community composition among all sites, with a 12.41 final stress. Axis 1 accounted for 57% of the total variation in composition and was correlated with nitrogen deposition ($R^2 = 0.32$), while Axis 2 described 34% of the total variation and was correlated with latitude ($R^2 = 0.89$) and longitude ($R^2 = 0.89$). Mean ordination scores for each site are shown in black, with SE bars shown for both axes. MRPP results indicate significant differences in composition among all sites: A = 0.3, all p values < 0.001.

N deposition (R^2-m = 0.001; **Table 2.2**). Including precipitation as either an additional or alternative predictor of dominant species cover did not improve the overall variation described by either species' model (**STable 2.2**).

Species diversity (H') was weakly negatively correlated with N deposition (R^2-m = 0.07; **Fig. 2.5b**; **Table 2.2**), and the amount of variation accounted for by the model was not improved by including both N deposition and annual precipitation (**STable 2.2**). The plant species composition of each site differed in ordination space (A = 0.3, all pairwise comparisons p < 0.001), and the NMDS visualization used a two dimensional solution, with a final stress value of 12.41, and an instability of less than 0.0001 (**Fig. 2.6**; Axis 1: 57% variance, Axis 2: 32%

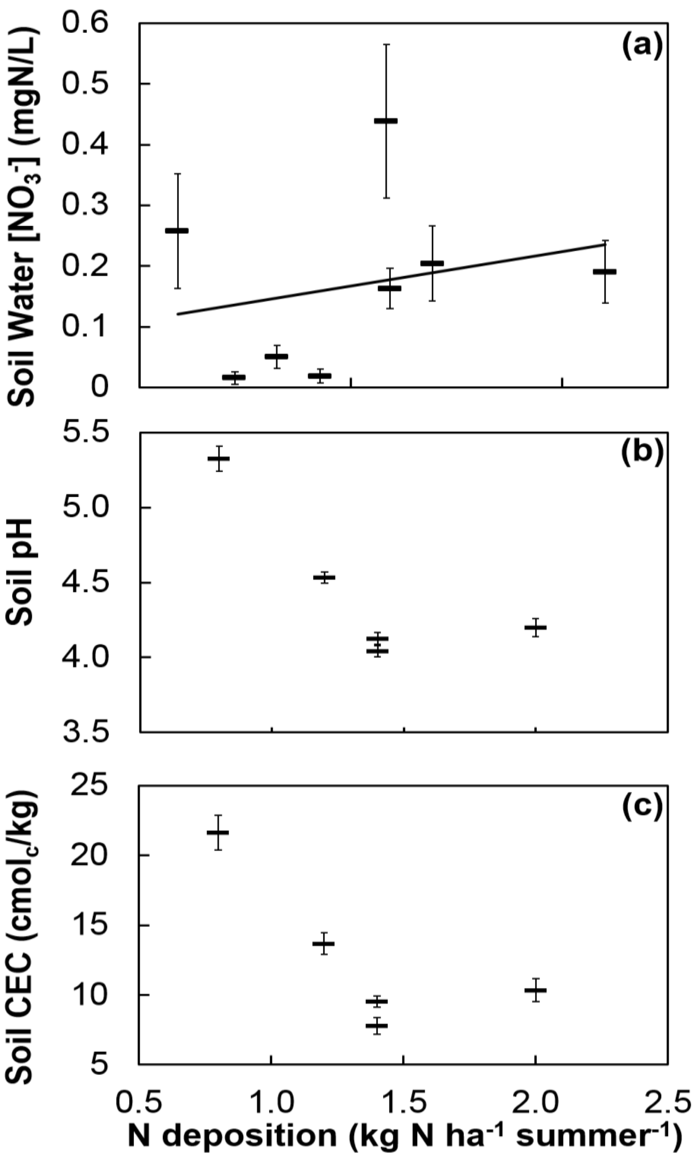

Figure 2.7 The relationship between summer N deposition and **(a)** soil pore water [NO_3^-], **(b)** soil pH and **(c)** soil cation exchange capacity (CEC). Points are means ± one standard error.

variance). Axis 1 was correlated most strongly with longitude (R^2 = 0.37), and N deposition (R^2 = 0.319), while Axis 2 was correlated with elevation (R^2 = 0.90), longitude (R^2 = 0.89), and latitude (R^2 = 0.89). Species richness was not strongly correlated with N deposition (R^2-m = 0.06; **Table 2.2**), consistent with my expectation that this plant metric would be slow to respond. Including both the long term site average precipitation and N deposition as fixed effects in the analysis improved the total variation described by the model (**STable 2.2**).

Ecosystem soil metrics of change

I examined a total of five different hypothesized soil metrics of ecosystem change, including two medium scale metrics (resin bag N, and pore water [NO_3^-]) and three slow scale metrics (soil pH, cation exchange capacity- CEC, and C:N). Of the medium scale soil metrics, resin extractable soil total N was not strongly correlated with N deposition (R^2-m = 0.03; **Table**

2.3). This relationship was not improved by including local precipitation as a predictor (**STable 2.3**). Soil pore water [NO_3^-] showed a moderate positive correlation with N deposition (R^2-m = 0.34; **Fig. 2.7a**; **Table 2.3**), and a significant effect size. Additionally, including both N deposition and precipitation as predictors accounted for greater variation in soil pore water [NO_3^-] than N deposition alone (**STable 2.3**).

Within the slow scale soil indicators (pH, CEC, and C:N), soil pH, and cation exchange capacity declined with increasing N deposition (**Table 2.3**; **Fig. 2.7b,c**) when all sites were included in the analysis, however this correlation was driven by differences in parent material source, as Shoshone had higher concentrations of minerals contributing to soil base cations than the other sites (**STable 2.1**). When Shoshone data were excluded from this analysis, no correlation was found between pH or CEC and ambient N deposition (**Table 2.3**), and these relationships were not improved by including annual precipitation (**STable 2.3**). Soil C:N was not correlated with N deposition (**Table 2.3**), however the addition of annual precipitation as a predictor explained more variation in soil C:N than N deposition alone (**STable 2.3**).

Table 2.3 Ecosystem soil metrics model effect size and total variation associated with nitrogen deposition

Ecosystem indicator	**Intercept**	**N Deposition (Measured)**	**R^2-m†**	**R^2-c‡**	**P Value**
Soil resin extractable N (mgN resin bag^{-1} $Summer^{-1}$)	0.54 ± 0.09	-0.08 ± 0.06	0.032	0.547	0.18
Soil pore water [NO_3^-] (mgN/L)	-6.61 ± 0.71	2.46 ± 0.26	0.339	0.825	<0.001
Soil pH	1.75 ± 0.08	-0.19 ± 0.06	0.412	0.914	0.005
Soil pH (no Shoshone)§	1.48 ± 0.08	-0.03 ± 0.05	0.028	0.738	0.54
Soil CEC ($cmol_c$/kg)	25.2 ± 4.2	-9.13 ± 2.87	0.338	0.782	0.008
Soil CEC ($cmol_c$/kg) (no Shoshone)§	11.9 ± 3.9	-1.19 ± 2.51	0.012	0.499	0.65
Soil C:N	12.1 ± 0.4	0.22 ± 0.25	0.029	0.345	0.39

†Marginal R^2- associated with fixed effects only

‡Conditional R^2- associated with both fixed and random effects

§Analyses performed excluding data from Shoshone

Discussion

By employing an ambient gradient of N deposition across the southern Rocky Mountain alpine, I was able to examine the relationship between low levels of N deposition and numerous potential ecological metrics of change. Based upon my proposed framework (**Fig. 2.1**), I hypothesized that increased N deposition would lead to increased cumulative ecosystem response, with correlations between N deposition and my fast and medium stage metrics. My results support a relationship between some of the fast and medium stage metrics and N deposition, indicating that moist meadows may be responding to N deposition along my study gradient. There were moderate correlations for growing season tissue concentrations of N, abundance of a nitrophilic grass species and a metrics of soil available N, as well as slight correlations with total ANPP and diversity.

Aboveground net primary production (ANPP) is one of the most common indicators used to quantify ecosystem response to changes in environmental drivers (Chapin et al. 2009, Isbell et al. 2015). For some N limited ecosystems increases in N availability may promote growth to a limited extent (Vitousek and Howarth 1991). This pattern holds true across numerous climate zones and types of ecosystems, and relatively low levels of ambient N deposition may increase ANPP (Sutton et al. 2008, de Vries et al. 2009, Stevens et al. 2015). Previous research in the alpine of the southern Rocky Mountains has shown that ecosystems experience nutrient limitations to production (N and phosphorus), but only at levels far exceeding current N deposition rates (Bowman et al. 1993, Suding et al. 2004) and associated with community composition shifts that reflect increases in species able to take advantage of the increased resources. More recent work in alpine communities using N additions between 5-60 kg N ha^{-1} yr^{-1} have not shown consistent increased ANPP with increased N both due to more subtle shifts in composition with the lower N inputs as well as strong inter-annual variability (Bowman et al.

2006, 2012). In my current study, the levels of N additions associated with ambient deposition were lower than those tested in previous fertilization studies (highest value 6.2 as compared to the 158 kg N ha^{-1} yr^{-1} added in earlier manipulations). Factors reducing the ability of plant production to increase associated with higher resource availability include key physiological and developmental constraints on plant growth in alpine plants associated with lowering N requirements for growth and survival (Billings 1974, Meloche and Diggle 2001, Korner 2003). As a consequence of these known constraints for a number of alpine plant species, the weak correlation found in this study between N deposition and total ANPP is not surprising.

I did find support that tissue concentrations of N may have increased in response to N deposition, suggesting luxury uptake of N by the dominant species and a potential physiological trade-off between new growth and the concentration of N in the tissues of that plant (Korner 1989, Hobbie 1992, Theodose et al. 1996, Monson et al. 2006). This pattern has been measured previously with simulated N deposition in other alpine areas (Bowman et al. 2006, 2012), and is supported by research on the growth constraints of some of the dominant species present in moist meadow communities (Korner 1989, Lipson et al. 1996, Meloche and Diggle 2001). Increased storage of N in tissues can function as a key feedback to N cycling through the deposition of litter (Hobbie 1992) however there was no relationship between N concentration in litter and N deposition in my study. As such, it appears that plants may not yet be providing amplifying feedbacks affecting ecosystem responses to N deposition across the Rocky Mountains.

I focused my research in the moist meadow community due to its high cover in the Southern Rocky Mountain alpine (40% of vegetated area, Komarkova, 1980), and the differences in N use by the two main dominant plant species (*D. caespitosa* and *G. rossii*). *Deschampsia caespitosa,* a grass, promotes fast rates of N cycling through rapid uptake and tissue turnover and stimulation of mineralization and nitrification (Steltzer and Bowman 1998, Miller and Bowman 2003, Bowman et al. 2004, Miller et al. 2007, Ashton et al. 2008, 2010, Meier and Bowman 2008). On the other hand *G. rossii*, a forb, enhances soil microbial biomass, which immobilizes N, therefore reducing the overall N available to the surrounding plant community and allowing *G. rossii* a competitive advantage (Bowman et al. 2004, Suding et al. 2006). Additionally, competitive interactions are modified by levels of N addition, favoring recruitment of *D. caespitosa* under increased levels of N availability (Suding *et al.*, 2004). Thus, I expected to see differential changes in the abundance of these two species under increasing N deposition. This hypothesis was supported by greater cover of *D. caespitosa* at sites receiving elevated N deposition, while the cover of *G. rossi* was not correlated with N deposition. When examining overall community composition using ordination, my first axis (54% of total variation) showed a moderate correlation with N deposition, suggesting that subdominant species are likely responding both to changes in N deposition and the relative presence of the dominant species as well.

While there was a positive correlation in the relative abundance of *D. caespitosa* and N deposition, I only saw a minor change in species diversity with N deposition, potentially due to my low levels of N inputs. A negative correlation between plant diversity and increasing N deposition ambient deposition is consistent with findings in ecosystems that span a higher range of N deposition levels or experimental N additions across the United States (Suding et al. 2005, Clark et al. 2013, Baron et al. 2014, Simkin et al. 2016), and across grassland communities in Europe (Stevens et al. 2006). On the other hand, a N deposition experiment in the alpine showed an increase in diversity associated with increased evenness of species (Bowman et al. 2006). In addition a survey of herbaceous communities across the continental U.S. indicated increases in species richness occur at low levels of N deposition, with a trend toward decreasing species richness above 10 kg N ha^{-1} yr^{-1} (Simkin et al. 2016). While I do not yet know the ecological implications for changes in diversity, I do know that any changes in alpine moist meadow communities are primarily driven by changes in the relative evenness among species that are present, and not associated with loss of rare species or establishment of non-native species as found in lower elevation US and European grasslands (Clark and Tilman 2008, Duprè et al. 2010, Maskell et al. 2010, Stevens et al. 2010b, 2010a). The lack of a strong relationship between species richness and N deposition supports this suggestion. Indeed, this result is consistent with experimental N additions in alpine communities that have altered diversity but have not produced any change in species richness, suggesting that at least under current levels of N deposition in the absence of other physical disturbances we are not likely to experience changes in plant slow stage metrics (Bowman et al. 2006, 2012).

The hierarchical soil metrics that I examined included resin extractable soil N and soil pore water concentrations of NO_3^-, as well as three slow stage metrics of ecosystem change (soil pH, cation exchange capacity, and bulk soil C:N). These metrics were selected to highlight changes in N cycling associated with increased N deposition. In this hierarchical framework, increased NO_3^- concentrations in pore water only occurs as soil N availability increases. In turn, the increased concentrations of NO_3^- in soil pore water promote leaching losses of base cations and this increased leaching reduces the buffering capacity of soils and leads to lowered pH over long periods of time if bedrock weather rates remain unaffected and N inputs remain elevated (Driscoll et al. 2003).

I found that while resin extractable N was only slightly correlated with increasing N deposition, there was a significant positive relationship between NO_3^- concentrations in pore water with increasing ambient N deposition. Experimental additions of N in the alpine have shown increases in both resin extractable N and pore water NO_3^-, however these changes were only observed at levels (> 15 kg N ha^{-1} yr^{-1}) higher than those experienced along my gradient (Bowman et al. 2006, 2012). On the other hand, lower inputs have prompted changes in nitrate leaching in forested catchments for areas receiving long-term chronic inputs of N deposition (Dise and Wright 1995), suggesting that incorporating metrics of cumulative N inputs is likely to be important in understanding changes in soil processing of N from deposition (Humbert et al. 2016).

I did not find support for a relationship between N deposition and any slow stage soil metrics, when the influence of bedrock composition was accounted for. Instead, the low amount of variation described by either N deposition or precipitation in my models suggests that local scale factors such as topography or soil texture likely play a more important role in determining variation for these soil metrics. Previous research in alpine communities have shown changes in soil chemistry in plots receiving high levels of N additions (> 20 kg N ha^{-1} yr^{-1}), or in sites where the soil buffering capacity is reduced associated with a legacy of acid rain (Bowman et al. 2008). Other ecosystems have experienced changes in soil pH under N addition levels greater than 20 kg N ha^{-1} yr^{-1}, however changes in pH generally only occur after chronic applications of N and may be associated with an accumulated input rather than a particular amount of N (Power et al. 2006). My results indicate that limited ecosystem responses to N deposition have occurred for soil metrics under the current total accumulation rates, however there is a future potential for feedbacks between plant and soil cycling of N that could facilitate continued elevated N processing (indicated by soil pore water nitrate and dominant species tissue N concentrations) unless reductions in total N deposition are implemented to prevent future ecosystem changes.

Alpine ecosystems are considered highly susceptible to environmental degradation with increases in N deposition (Billings 1974, Nanus et al. 2008, Williams et al. 2009). Consequently, this ecosystem was ideal to examine the potential effects of low levels of ambient N deposition, and in particular the changes in potential indicators associated with plant-soil feedbacks, such as nutrient uptake or storage and turnover by plants, that may promote or prevent long-lasting consequences of N deposition (Hobbie 1992, Chapin et al. 1997). Changes in composition align with results from previous studies (Bowman et al. 2006, 2012), while changes in diversity match findings from continental scale N deposition gradient analyses. There are many known ecosystem consequences from increased N deposition, and ongoing research is establishing thresholds associated with how much N may be added to an ecosystem before irreversible change occurs (Clark and Tilman 2008, Gaudnik et al. 2011). Which ecosystem metrics to use for these thresholds, however, has not been thoroughly addressed and determining which metrics to use as indicators of ecosystem changes in an integrated framework (Fig. 1) enables comparisons among studies using different types of metrics. My results suggest that, for the alpine, metrics such as growing season tissue chemistry and abundance of dominant plant species are best suited to track the consequences of N deposition across a broad spatial area. Furthermore, my results show that current ambient deposition in some areas of the Rocky Mountains are high enough to prompt changes in ecosystem metrics predicted to change with increased N availability from deposition.

CHAPTER 3

Alpine community dependent responses and ecological thresholds to elevated nitrogen deposition in the Rocky Mountains

Abstract

Community scale processes can provide stabilizing feedbacks in maintaining ecosystem function in the face of environmental changes. However, less experimental work has included comparisons across communities as a means of including variation in plant and soil responses that contribute feedbacks to ecosystem reactions to global change. Elevated levels of nitrogen (N) deposition have been implicated in landscape scale ecosystem modifications and subsequent changes in N availability have the potential to alter existing plant-soil feedbacks. In this study, I compared a suite of ecological response metrics for both plants and soils to relatively low dosage levels of experimental N additions (0, 3, 12, and 30 kg N ha^{-1} yr^{-1}) among three alpine communities (dry, moist, and wet meadows) to examine variation in responses that may contribute feedbacks associated with ecosystem change. These communities include contrasting potential feedbacks to ecosystem changes with N deposition including species diversity, resource-use species traits and ambient resource availability. Based on my findings, dry meadow communities, with high levels of plant diversity and low ambient resource availability, showed the strongest response to N additions with strong changes in metrics associated with a dominant sedge species *Carex rupestris*. Moist meadow communities, with low species diversity and higher resource availability, were less strongly affected by N addition despite dominance of a grass species previous identified as responding strongly to increased N inputs. Finally, the wet meadow showed the only evidence for increased productivity with N inputs, as well as increased foliar N concentrations for one dominant forb species, and increases in cover for the other dominant forb species. I found that the dry meadow communities generally showed the lowest ecological thresholds associated with plant-soil response metrics, including an increase in

aboveground tissue concentrations of N (15 kg N ha^{-1} yr^{-1}), increases in species diversity (> 34.6 kg N ha^{-1} yr^{-1}) and changes in resin extractable soil N (32.2 kg N ha^{-1} yr^{-1}) after three years of low dosage N additions. Furthermore, my results support the application of critical loads measured in alpine dry meadows as guidelines associated with ecosystem change in the Rocky Mountains.

Introduction

Terrestrial plant communities can both respond to changes in abiotic conditions, such as climate change or nitrogen (N) deposition, as well as modify the abiotic conditions in which they are found through feedbacks to ecosystem processes. There are many ways that communities exert influence on ecosystem processes, and studies commonly focus on weighted measures of species traits or influences driven by interactions among species as biotic feedbacks (Chapin et al. 1997, 2000, Hooper 1997, Hooper et al. 2005). Such biotic feedbacks at the community scale are hypothesized to contribute to differences in resistance and resilience among ecosystems in the face of disturbance or environmental change (Chapin et al. 2000, Elmqvist et al. 2003, Lloret et al. 2012, Loreau and de Mazancourt 2013, Wang and Loreau 2016). Additionally, biotic feedbacks have the potential to determine the magnitude and rate of ecological change associated with environmental variation (Chapin et al. 1997) and influence ecological thresholds linked with changes in ecosystem function (Groffman et al. 2006).

Nitrogen deposition is an important environmental change factor affecting community-ecosystem processes through shifts in resource driven species interactions and trait selection within a community that influence plant control on N cycling (Stevens et al. 2004, Bowman et al. 2006, Clark and Tilman 2008, Simkin et al. 2016). The rapid increases in N deposition associated with agricultural and industrial development (Galloway et al. 2004, Holland et al.

2005) have necessitated the need for understanding variation in plant and soil responses within ecosystems to aid management goals limiting widespread ecosystem change (Porter et al. 2005). As communities are the scale at which feedbacks between plants and soils affect ecosystems (Hobbie 2015), community differences in plant and soil responses to N deposition have important implications for changes in rates of N cycling within an ecosystem experiencing N deposition. For example, communities located in ecosystems with high resource availability and containing species capable of responding to changes in nutrient availability may respond to changes in nutrients more strongly than communities where species are physiologically constrained due naturally low resource availability (Billings 1974, Hobbie 1992, Korner 2003). These community differences driven by underlying variation in resource availability can in turn drive differences in biotic feedbacks to ecosystem processing of N, which has important implications for future ecosystem change.

Under conditions of increased N deposition, many plant and soil N pools may change and the amount of added N necessary to induce this change (ecological threshold) creates a hierarchy of expected ecological responses with increasing N deposition (Aber et al. 1989, Smith et al. 2009, Churchill et al. in review). In plants, initial changes in N pools include alternations in plant allocation to growth and N storage (physiological responses), followed by shifts in species abundances within the community (evenness responses), before finally producing changes in species presence (species change responses) (Churchill et al. in review., Smith et al. 2009, Lovett and Goodale 2011). For soils, increased N inputs also alter soil processing of N, which can be measured by changes in pools such as resin extractable N as well as soil pore water nitrate (NO_3^-) concentrations (soil processing responses). Increases in these soil N pools can promote leaching of soil cations and soil acidification over long time intervals or high accumulation of soil N (soil chemistry responses; Vitousek et al. 1997, Aber et al. 1998, Fenn et al. 2003a, Bowman et al. 2008). Ecological thresholds in plant and soil responses to N deposition suggest that shifts in plant community composition can occur with deposition levels between 5-20 kg N ha^{-1} yr^{-1} (Bobbink et al. 2010), while shifts in individual species abundances are usually at lower input rates (Bowman et al. 2006). Changes in the evenness and richness of a community have been measured above 9-15 kg N ha^{-1} yr^{-1} (Suding et al. 2005, Bobbink et al. 2010, Clark et al. 2013, Simkin et al. 2016). Additionally, changes in production and leaching of NO_3^- from soils have been measured at N deposition rates greater than 10 kg N ha^{-1} yr^{-1} in ecosystems across North America and Europe (Dise and Wright 1995, Fenn et al. 1998, Bowman et al. 2012) and declines in buffering capacity and increases in acidity of soils above 40 kg N ha^{-1} yr^{-1}(Bowman et al. 2008, 2014).

Some ecosystems are sensitive to N deposition and experience low ecological thresholds associated with ecosystem change due to smaller sinks capable of stabilizing reactive N in plant biomass and soils (Bowman et al. 2014). The alpine of the southern Rocky Mountains is at high risk for potential ecosystem change due to increases in agricultural and industrial development and associated increase in the rates of N deposition (Porter and Johnson 2007, Benedict et al. 2013b, Bowman et al. 2015). In the alpine, short growing seasons limit plant uptake of N, and physiological constraints on species frequently limits increased growth (Bowman 2000, Meloche and Diggle 2001) except under high levels of total accumulated N inputs driving shifts in community composition (Bowman et al. 2006, Gasarch and Seastedt 2015, Farrer and Suding 2016). Shallow and poorly weathered soils additionally limit accumulation of soil pools of N (Bobbink et al. 2010, Bowman et al. 2014), and spring pulse release of winter accumulated N in the snowpack further limits the temporal window for biotic stabilization of added N (Bowman

1992, Williams and Baron 1996, Darrouzet-Nardi et al. 2012). Slight alterations in the plant and soil pools of N with increased N inputs can therefore have large implications for ecosystem change in the alpine, and the variation in responses among communities may be important for predicting long-term consequences. Numerous studies have highlighted potential implications of elevated N deposition in this area (Baron 2006, Bowman et al. 2006, 2012, Gasarch and Seastedt 2015, Farrer and Suding 2016). However, no studies have explicitly compared community differences among plant and soil responses to N deposition despite evidence for strong biotic feedbacks on ecosystem processing of N (Bowman 2000).

Taking advantage of the range in potential plant and soil responses to N deposition among different alpine meadow communities, my research addressed two main questions focused on understanding how three communities respond to low levels of N deposition: 1) how do alpine dry, moist, and wet meadow communities differ in plant and soil responses to N deposition? 2) Do communities differ in their thresholds for change in plant and soil responses to N deposition? I hypothesized that differences in ambient resource availability and plant traits among communities will facilitate paired responsiveness in plant and soil metrics with increased N deposition. Communities will therefore differ in these responses based on the abundance and response magnitude for species able to take advantage of increased N and the strategy those species use. I predicted that responsive traits are more common in communities with greater resource availability creating a pattern of moist > wet > dry meadow community responses to N deposition. Variation in resource availability will also likely be important in determining differences in soil N processes responses among communities, coupled with tradeoffs between plant and soil uptake of N. Communities with strong plant responses are therefore more likely to see elevated cycling of N between plants and soil, by increasing pools of biologically active N, while communities with high water availability may facilitate the rapid loss of added N from the ecosystem (moist> dry> wet meadow communities).

Methods

Field site

My research site is located in the Fool Creek watershed at Fraser Experimental Forest (Fraser), in Arapaho National Forest outside the town of Fraser, CO, USA. Elevation at the field site ranges from 3597 m to 3612 m. Average annual temperature at the Fraser headquarters (elevation 2750 m) is 0.55 °C (1976-2003), with an average July temperature of 12.4 °C, and an average January temperature of -10.0°C. Annual average precipitation is 860 mm, and the monthly highest precipitation occurs in April, May, and March respectively (Elder 2005, 2006).

I measured ambient bulk N deposition during the growing season using passive ion-exchange resin column deposition samplers (Bytnerowicz et al. 2001, Fenn et al. 2003b). Additionally, site based measurements of wet deposition are made by the National Forest Service at the Fraser headquarters, with an average rate of 2.5 kg N ha^{-1} yr^{-1} between 1996- 2007 (Argerich et al. 2013). For addressing my research questions examining the effect of N deposition on plant and soil response metrics among alpine communities, I used a combined value of long-term average wet annual deposition plus annual dry deposition as estimated based on the Community Multiscale Air Quality (CMAQ) model (2.1 kg N ha^{-1} yr^{-1}; Simkin et al. 2016) for a total ambient N input of 4.6 kg N ha^{-1} yr^{-1}.

Experimental design

I established N treatment plots at Fraser in the summer of 2013 for each of three alpine plant communities, including dry, moist and wet meadows. These communities represent 20, 20, and 3% cover across a regionally similar alpine area at Niwot Ridge, and therefore are representative of common vegetated alpine areas of the southern Rocky Mountain (Komarkova and Webber 1978, Komarkova 1980, Bowman et al. 1996). Communities were identified based on presence of dominant plant species (May et al. 1982, Walker et al. 1993, 1994, Spasojevic et al. 2013; with dry meadows dominated by *Kobresia myosuroides* and *Carex rupestris*; moist meadows dominated by *Deschampsia caespitosa* and *Geum rossii*; and wet meadows dominated by *Caltha leptosepala* and *Sibbaldia procumbens*). Wet meadows at Fraser are similar to a subset of snowbed communities also present at Niwot Ridge, associated with a broader categorization of the community types for this classification and a range in the types of species assemblages present across the alpine. Winter snow cover is the primary driver of a hydrologic gradient among these communities (Walker et al. 1993, 2001b, Gasarch and Seastedt 2015). Additionally, environmental resource availability, including N and water, are lowest in the dry meadow communities while moist meadows have higher water and N supplies associated with depositional inputs and storage of N in winter snowpack and subsequent runoff (Bowman 1992, Walker et al. 1993, Fisk et al. 1998). Saturated soils in wet meadows may facilitate high rates of denitrification during the early part of the growing season, which results in low soil N availability (Darrouzet-Nardi and Bowman 2011), although the soil retains soil moisture longer into the growing season facilitating potential late season belowground growth (Fisk et al. 1998). Fertilization studies conducted among these communities have determined that primary production is limited by N in dry and moist meadows, while wet meadows are N and phosphorus (P) co-limited (Bowman et al. 1993, 2006, 2012, Bowman 1994, Theodose and Bowman 1997). Species diversity also varies among these communities, with dry and wet meadow communities having higher vascular plant diversity and the moist meadow communities comparatively low diversity (Walker et al. 1993, 2001b, Gasarch and Seastedt 2015). Finally, dry and moist communities are dominated in cover by two different sets of species with contrasting resource-use traits, which have important implications for plant-soil feedbacks (Steltzer and Bowman 1998, Bowman et al. 2004, 2006, Suding et al. 2006, Miller et al. 2007, Ashton et al. 2010). Many of these community dominants have been the subject of extensive research examining functional traits from alpine areas in the Rocky Mountains (Raab et al. 1996, Steltzer and Bowman 1998, Walker et al. 2001a, Miller et al. 2007, Ashton et al. 2010), including data on N uptake preferences, competitive relationships with other community members, and litter impacts on nutrient cycling that are important for plant-soil feedbacks.

I established 1 m by 1.5 m treatment plots in each community, including a 1 m by 1 m undisturbed section used for repeated non-destructive measurements of plant composition cover and a 1 m by 0.5 m portion for destructive measurements (Bowman et al. 2006). I avoided inclusion of N_2-fixing species (predominantly *Trifolium parryi*) during plot placement to minimize the heterogeneity of soil and plant N associated with biological N_2 fixation. Each community type contained five blocks of four plots, with each plot treated with one of four levels of N addition levels (0, 3, 12, 30 kg N ha^{-1} yr^{-1}; control, low, medium, high treatments; based on known ecological thresholds for alpine dry meadows from Bowman et al. 2012) for a total of 60 plots. I applied N treatments as NH_4NO_3 in solution using a backpack sprayer at three times during the growing season. I added half the total amount in early spring to simulate the N influx associated with snow melt and the remaining half divided between applications at peak biomass and post plant senescence (Bowman et al. 2006, 2012). Control plots received the same amount of water (2 L) with no dissolved N. The control treatment is equivalent to an increase in total precipitation of 4 mm yr^{-1} and falls within 0.7 % of the long term precipitation average for

Fraser. All water for applications was obtained from a first order stream located within 0.25 km from the alpine communities, with total dissolved inorganic N in this source measured during each application period (Mean: 0.54 mg N L^{-1}, Range: 0.002-2.96 mg N L^{-1}).

Soil texture was measured at the Soil, Water, and Plant Testing Laboratory at Colorado State University, from samples collected directly adjacent to treatment plots during the summer of 2014. Additionally, I collected bulk soil samples from each plot in the summer of 2013 and used subsamples to measure soil pH and cation exchange capacity (CEC). Soil pH was measured on two to one water to soil mixtures, after 30 seconds of shaking and a 30 minute rest, using a Beckman 340 pH probe. I measured CEC using 0.1 M $BaCl_2$ extractions of cations, including Al^{3+}, Ca^{2+}, Fe^{3+}, K^+, Mg^{2+}, Mn^{2+}, and Na^+ measured using an ARL Inductively Coupled Plasma Emission Spectrophotometer (LEGs Laboratory, University of Colorado Boulder).

Measurements of plant and soil responses to n additions

I measured multiple potential plant and soil responses to N additions among alpine communities to address my first research question, including metrics for plant physiological responses, plant evenness responses, plant richness responses and soil pools of N responses to N additions. These metrics align with previous research in ecosystems responding to N deposition in examining hierarchical ecological indicators of change (Churchill et al. in review, Aber et al. 1998, Smith et al. 2009). For plants, metrics included both above and below-ground net primary production for the entire community and the two dominant species within each community (physiological response), concentrations of N in aboveground biomass and litter (physiological response), abundance for dominant plant species, plant community composition, species diversity, and richness (evenness and species losses or gains responses). Soil metrics included resin bag extractable soil N and soil pore water concentrations of nitrate ([NO_3^-]; soil N pool responses).

As my first plant physiological response I estimated both above and belowground net primary production (ANPP, BNPP) in response to increased levels of N addition. Aboveground NPP was measured by harvesting all biomass in 20 cm by 20 cm subplots from each plot at peak biomass in the summer of 2015 (year 3 of study). I then sorted these samples to separate new growth from previous year litter, and further subdivided new growth into dominant plant species and dried all samples at 60° C to a constant mass before weighing. I measured BNPP using two root in-growth bags installed into control and 30 kg N ha^{-1} yr^{-1} treatment plots in each community during October 2014 (intermediate N addition treatments were not included due to processing constraints). Root in-growth cores were constructed of nylon stockings filled with root-free soil from the same community, although the soil used to fill stockings was not previously exposed to N additions. Previous research at Niwot Ridge, a compositionally similar alpine ecosystem, has shown that 80-90% of roots occur within the upper 15 cm in these communities (Webber and Ebert-May 1977). I therefore installed in-growth bags as close to this depth as possible although there was some variation due to differences in soil rockiness among communities. Dry meadow in-growth bags were approximately 9 cm deep while moist meadows were on average 13 cm deep and wet meadows averaged 14 cm deep. Cores were removed in late August 2015. Root in-growth cores were then frozen (0° C) until thawed for root separation from soil (range 3-11 months). Thawed in-growth cores were measured for exact length, width, and height to determine soil volume and protruding roots were clipped from the outside of each bag. Roots within a core were removed to achieve a consistent density of remaining root hair fragments across subsamples and between cores. All roots were rinsed with water to remove soil particles and dried in an oven at 60° C to a constant mass before being weighed to determine BNPP.

As my second plant physiological response to N deposition, I measured aboveground plant tissue concentrations of N ([N]) and collected aboveground tissue samples of the dominant plant species from each plot at peak biomass (biomass, late July) and following senescence (litter, early Sept.) in the summer of 2013 (first year of study) and 2015 (third year of study). These samples were oven dried to a constant mass and ground and homogenized using a mortar and pestle with liquid N before analysis of the [N] present in plant tissues using a CHN autoanalyzer for elemental analysis (Flash 1112 Series).

To examine differences in plant evenness and species losses or gains responses among communities in response to N deposition, I measured plant community composition using the point-intercept method with a 10 x 10 grid of 100 points in a 1 m by 1 m portion of each plot. Total vegetation cover in each plot could be greater than 100% associated with multiple species present at each grid point. For species that were present in the plot but not recorded within the grid points, I assigned a projected cover value of 0.5%. I used vascular plant species abundance values to calculate species richness (species losses or gains response) and diversity (species evenness response; Shannon Diversity index) for each plot from 2013-2015. Differences in community composition as a multivariate response to N deposition was also examined as a species evenness response metric as described below. Vascular species nomenclature followed the USDA Plants database (http://plants.usda.gov), while non-vascular cover was described only to functional group (moss, lichen).

To determine the effect of increased N inputs on soil pools of N among communities, I measured resin extractable soil N as an estimate of growing season soil N availability and soil pore water concentrations of nitrate ([NO_3^-]) as a point measurement of mobile N following snow melt. I used mixed bead resin bags buried within the root zone to capture vertical transport and production of N (upper 10 cm) during growing season months of 2013 and 2015 (year 1 and year 3 of the study), with 1 resin bag deployed per plot (60 total). Resins were extracted using 2 M KCl, and analyzed for NO_3^- and NO_2^-, using a Lachat QuikChem 8000 Specto- Flow Injection Module (Bowman et al., 2012), and NH_4^+ using a BioTek Synergy 2 Multi-Detection Microplate Reader. I collected water used for [NO_3^-] measurements using Rhizon soil lysimeters installed in each treatment plot at 10 cm depth for all three communities (60 total). Soil pore water samples were collected within two weeks of snow melt and prior to fertilization of the treatment plots (Bowman et al., 2012). These samples were collected in 2013- 2015. Soil pore water was analyzed for total concentration of NO_2^- and NO_3^- as described above.

Data analyses

My first research question focused on comparing the response of various plant and soil metrics to N deposition among alpine communities. To address this question, I used mixed effects models to examine the relationship between additions of N and univariate plant and soil responses for each alpine communities using the R package nlme (Pinheiro et al. 2014). These models included total N inputs (ambient N deposition plus N added as fertilization) as a continuous fixed effect and block as a random effect. For metrics with multiple years of data (such as plant diversity), I included year as random effect to account for repeated measures sampling within a plot. For the analysis of BNPP, which included two in-growth cores per plot, I also included plot as a random effect. Amount of variation associated with each linear mixed model was reported using marginal R^2 (R^2-m: variation ascribed to fixed effects component of model) and conditional R^2 (R^2-c: variation described by the full model) using the MuMIn package (Barton 2015). All response metrics were examined for assumptions of normality prior to analysis, and transformations were applied as necessary to meet model assumptions. Applied transformations included a log transformation for soil water [NO_3^-] and a 4th root transformation for resin extractable soil N.

For comparing multivariate plant responses to N deposition among communities (vascular plant community composition), I used a Non-metric Multi-Dimensional Scaling (NMDS) ordination with a Bray-Curtis dissimilarity metric to visualize differences in vascular composition among treatments through time within each community (evenness response). I compared the composition among treatments within a community using a PerMANOVA hypothesis test, also using the Bray-Curtis dissimilarity index. All vascular species present in greater than 2% total cover were included in the dissimilarity matrix, and I applied a relativization by maximum to species abundance to account for high coefficient of variance (CV) among species within a plot. Axes selection for the NMDS ordinations were based on Monte-Carlo simulations comparing stress between real data (250 runs) and randomized iterations of data (250) to find the best solution minimizing both total stress and number of axes (‘Slow and Thorough’, PC Ord 5.3.1; MJM Software). To examine the relationship between total N inputs and variation in vascular community composition, I determined the Pearson correlation of total N inputs with each NMDS axis.

To address my second research question, examining differences in ecological thresholds for plant and soil responses to N deposition among communities in the alpine, I first modeled the relationship of individual metrics in response to N. For models with a significant response to N I determined ecological thresholds associated with a change as compared to response values under zero N input conditions (**Figure 3.1**). I used linear mixed effects models associated with each plant and soil response metric to create models in response to N deposition for the third year of my study (2015) including total N inputs as a fixed effect (as described above) and block as a random effect. Models including multiple years of study potentially underestimated the lagged ecosystem responses of indicators to additions of N, and consequently I calculated the ecological threshold for indicators based on measurements of samples collected in year three of the study only. To determine ecological thresholds based on the different response metrics relationship with N deposition I created predicted estimates for each metric based on the associated linear mixed models. I then bootstrapped a 95% confidence interval surrounding the predicted values associated with each model using the R package lme4 (Bates et al. 2015). The ecological thresholds were then determined as the level of N (predictor) at which there was no overlap between the confidence intervals of a model and the model intercept (predictor = 0) (**Figure 3.1**; Bowman et al. 2006, 2012). The intercept value represents the plant or soil response assuming no N inputs or a statistical null hypothesis of no response with increased N input.

Results

Site background measurements

At Fraser I found that the growing season (June- August) bulk N deposition for 2013-2015 was 1.7, 0.7, and 0.6 kg N ha^{-1} 92 $days^{-1}$, respectively. Background soil measurements in the alpine meadows where I established the fertilization experiment showed that the dry meadow had a clay loam texture, while the moist meadow soils were sandy loam and the wet meadow soils were sandy clay loam (**Table S3.1**). Additionally, the dry meadow community had the highest average pH, with the moist meadow intermediate and the wet meadow the lowest; cation exchange capacity followed this same pattern (**Table S3.1**).

Plant and soil responses to N fertilization

I examined differences in how alpine meadow communities respond to elevated N deposition by comparing a total of fifteen different plant and soil response metrics hypothesized to provide differential contributions to ecosystem change. These included metrics of plant physiological responses (total above and belowground net primary production (ANPP and BNPP), aboveground production associated with dominant species in each community, aboveground tissue concentrations of N for dominant species during peak biomass and following senescence), plant evenness and species losses or gains responses (community species composition, dominant species cover, community diversity, and richness), and soil N processing responses (resin extractable soil N, and soil pore water concentrations of NO_3^-).

Table 3.1. Relationship between total N input with plant community and soil indicators of ecosystem responses

Community	Indicator	Intercept	N Addition	R^2-m[#]	R^2-c[^]	P Value
Dry	ANPP[*]	87.9 ± 14.4	0.737 ± 0.730	0.051	0.054	0.329
	Diversity	1.64 ± 0.10	0.007 ± 0.003	0.067	0.404	0.014
	Richness	17.8 ± 1.03	0.046 ± 0.036	0.021	0.229	0.208
	Resin extractable soil N[‡]	0.21 ± 0.07	0.012 ± 0.003	0.225	0.586	<0.001
	Soil pore water [NO_3^-][†]	-4.73 ± 0.36	-0.006 ± 0.008	0.011	0.858	0.721
Moist	ANPP[*]	248.9 ± 13.9	-1.25 ± 0.70	0.142	0.142	0.099
	Diversity	1.62 ± 0.06	0.005 ± 0.001	0.076	0.549	0.003
	Richness	13.23 ± 0.54	0.006 ± 0.015	0.002	0.446	0.686
	Resin extractable soil N[‡]	0.36 ± 0.08	0.008 ± 0.003	0.093	0.540	0.014
	Soil pore water [NO_3^-][†]	-4.88 ± 0.36	0.003 ± 0.008	0.015	0.561	0.716
Wet	ANPP[*]	122.9 ± 12.7	-0.203 ± 0.64	0.005	0.005	0.757
	Diversity	2.28 ± 0.08	-0.001 ± 0.001	0.002	0.571	0.635
	Richness	18.06 ± 0.80	-0.014 ± 0.024	0.004	0.333	0.554
	Resin extractable soil N[‡]	0.13 ± 0.08	0.007 ± 0.002	0.051	0.893	0.001
	Soil pore water [NO_3^-][†]	-4.98 ± 0.50	0.006 ± 0.017	0.005	0.324	0.442

[#] Marginal R^2- variation associated with fixed effects only (total N inputs)
[^] Conditional R^2- variation associated with both fixed and random effects (i.e. block effect and year)
[*] ANPP measured in (g/m^2)
[‡] Fourth root transformed resin bag N measured in mg N resin bag^{-1} 92 $days^{-1}$
[†] Ln transformed soil pore water [NO_3^-] measured in mg N/L

After three years of N addition manipulation, there was no effect of N addition on ANPP in both dry and wet meadow communities (Dry: $p = 0.33$; Wet: $p = 0.76$; **Table 3.1**). In the moist meadows there was a trend for declining ANPP with additions of N, although this relationship was not significant (p = 0.10; **Figure S3.1**; **Table 3.1**). Furthermore, I found no change in ANPP with increased N inputs for dominant species among all three community types (**Table 3.2**).

Table 3.2. Relationship between total N input and dominant plant species indicators of ecosystem responses

Community	**Indicator**	**Intercept**	**N Addition**	**R^2-m**[#]	**R^2-c**[^]	**P Value**
Dry	*C. rupestris* ANPP[*]	2.92 ± 2.79	0.120 ± 0.14	0.036	0.036	0.411
	K. myosuroides ANPP[*]	45.6 ± 11.1	0.124 ± 0.56	0.003	0.003	0.829
	C. rupestris Tissue N%	1.80 ± 0.10	0.018 ± 0.004	0.293	0.575	<0.001
	K. myosuroides Tissue N%	2.74 ± 0.17	0.003 ± 0.003	0.012	0.528	0.347
	C. rupestris Litter N%	1.06 ± 0.10	0.017 ± 0.003	0.213	0.895	<0.001
	K. myosuroides Litter N%	1.75 ± 0.09	0.004 ± 0.002	0.050	0.729	0.038
	C. rupestris Cover	8.06 ± 2.90	0.311 ± 0.076	0.147	0.483	<0.001
	K. myosuroides Cover	46.53 ± 5.09	-0.187 ± 0.139	0.019	0.379	0.186
Moist	*D. caespitosa* ANPP[*]	138.0 ± 11.4	-0.457 ± 0.58	0.032	0.032	0.442
	G. rossii ANPP[*]	61.07 ± 16.6	-1.09 ± 0.84	0.081	0.081	0.216
	D. caespitosa Tissue % N	2.54 ± 0.12	0.002 ± 0.004	0.003	0.483	0.589
	G. rossii Tissue %N	2.43 ± 0.06	0.002 ± 0.003	0.008	0.073	0.562
	D. caespitosa Litter N%	1.58 ± 0.06	0.002 ± 0.002	0.010	0.232	0.369
	G. rossii Litter N%	1.34 ± 0.07	0.006 ± 0.003	0.031	0.596	0.044
	D. caespitosa Cover	81.24 ± 3.00	-0.165 ± 0.095	0.021	0.472	0.089
	G. rossii Cover	28.16 ± 4.22	-0.158 ± 0.102	0.022	0.461	0.127
Wet	*C. leptosepala* ANPP[*]	7.68 ± 1.92	-0.033 ± 0.09	0.006	0.149	0.710
	S. procumbens ANPP[*]	12.40 ± 6.07	0.003 ± 0.308	<0.001	<0.001	0.992
	C. leptosepala Tissue N%	2.29 ± 0.07	-0.001 ± 0.003	0.003	0.219	0.807
	S. procumbens Tissue N%	2.31 ± 0.07	0.002 ± 0.003	0.014	0.163	0.467
	C. leptosepala Litter N%	1.22 ± 0.12	0.005 ± 0.002	0.016	0.873	0.041
	S. procumbens Litter N%	1.61 ± 0.13	-0.001 ± 0.002	0.001	0.894	0.563
	C. leptosepala Cover	6.10 ± 1.44	0.009 ± 0.037	0.0005	0.402	0.819
	S. procumbens Cover	12.21 ± 3.49	0.288 ± 0.087	0.095	0.497	0.002

[*] ANPP measured in (g/m^2)
[#] Marginal R^2- variation associated with fixed effects only (total N inputs)
[^] Conditional R^2- variation associated with both fixed and random effects (i.e. block effect and year)

Belowground I found a similar null result with increased N inputs in both the dry ($F_{1,9}$ = 0.73, p = 0.42) and moist meadow communities ($F_{1,9}$ = 0.53, p = 0.49). However, in the wet meadow community I found a significant positive effect of N addition on BNPP ($F_{1,9}$ = 5.24, p = 0.05), with high N treatment plots containing approximately twice the belowground growth of unfertilized plots (**Table 3.3**).

Table 3.3. Belowground net primary production measured using in-growth cores among alpine meadow communities for control and high N addition treatments

Community	**Control treatment***	**High treatment***
Dry meadow	283.4 ± 103.6a	204.7 ± 25.7a
Moist meadow	591.9 ± 111.7a	658.2 ± 95.0a
Wet meadow	545.6 ± 81.5b	964.2 ± 179.6a

* Measured in g/m^3, values are treatment means ± 1 standard error and same letter designations indicate non-significant differences within a community

I measured dominant plant species aboveground tissue concentration of N during the first and third years of the study, both during peak biomass and then again following senescence, as a physiological plant response associated with plant-soil feedbacks that may differ among alpine communities. In the dry meadow community tissue concentrations of N in *C. rupestris* collected at peak biomass significantly increased with N additions, with N inputs accounting for 29% of the total variation in tissue concentrations of N (**Figure 3.1A**, **Table 3.2**, $p < 0.001$). This species was the only dominant responsive to N inputs with a change in growing season tissue N concentration, however, as I did not find a relationship between total N inputs and biomass N concentration for the dry meadow species *K. myosuroides* ($p = 0.35$; **Table 3.2**), nor moist meadow dominant species *D. caespitosa* ($p = 0.59$) and *G. rossii* ($p = 0.56$; **Table 3.2**), and wet meadow dominant species *C. leptosepala* ($p = 0.81$) and *S. procumbens* ($p = 0.47$; **Table 3.2**). On the other hand, tissue concentrations following senescence were more responsive to N inputs and I found that litter concentrations of N were increased by total N inputs for four of the six species examined across the three communities (**Table 3.2**). In the dry meadow communities total N inputs significantly increased the concentration of N in litter for *C. rupestris* (**Figure 3.1B**; **Table 3.2**; $p < 0.001$) and *K. myosuroides* (**Figure 3.1C**; **Table 3.2**; $p = 0.04$). In the moist meadow the grass species showed no response (*D. caespitosa*; $p = 0.37$; **Table 3.2**), while forb *G. rossii* litter N

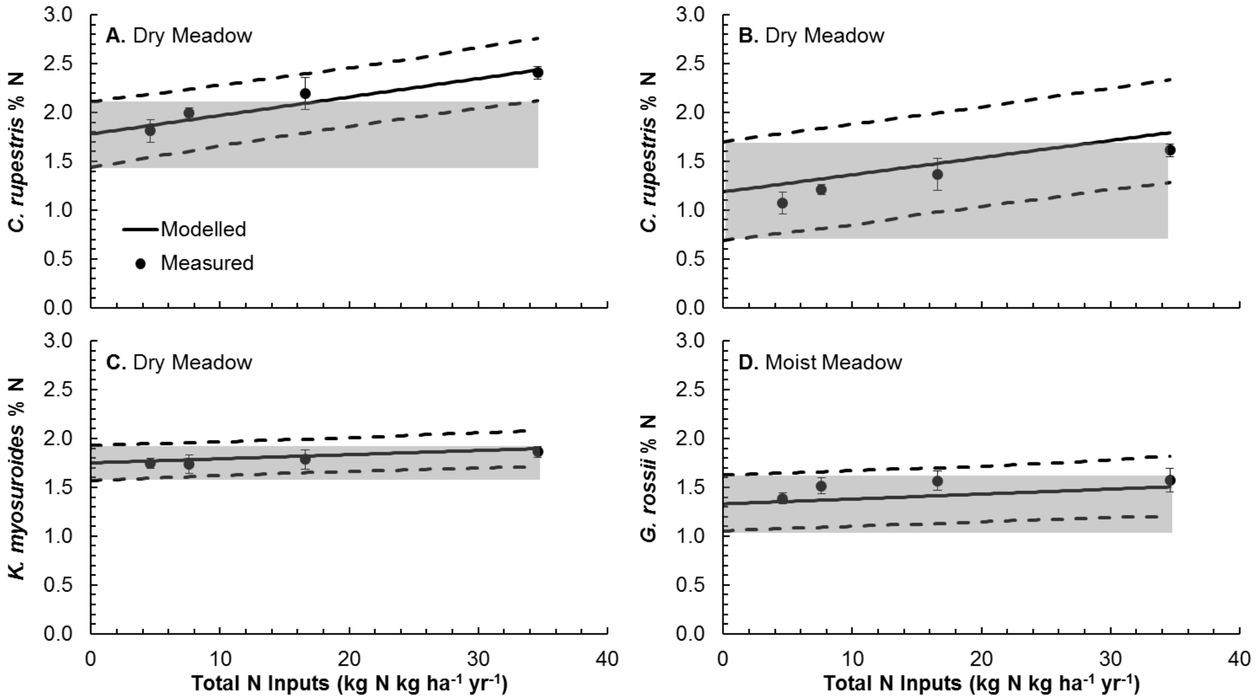

Figure 3.1. The effect of increasing total inputs of N on tissue concentrations of nitrogen for community dominant plant species **A.** *C. rupestris* at peak biomass, and following plant senescence for **B.** *C. rupestris*, **C.** *K. myosuroides*, and **D.** *G. rossii* (samples collected 2013, 2015). Points represent measured treatment means ± 1 SE, while the black line indicates modelled values from the linear mixed effects model including a 95% confidence interval (CI) outlined with the dashed black line. The level of total N inputs associated with the critical load occurs when the 95% confidence interval for the intercept (grey box) and the 95% CI for the model do not overlap.

concentration increased with inputs of N ($p = 0.04$; **Figure 1D**; **Table 2**). Wet meadow species *C. leptosepala* increased litter N with greater N inputs ($p = 0.04$), although *S. procumbens* did not respond to N ($p = 0.56$; **Table 3.2**).

For my multivariate species evenness response metric, I found that the variation in vascular species composition among treatments and years were best described by three main NMDS ordination axes for all three communities (Dry: 19.9 final stress, 73.6 CV; Moist: 18.9 final stress, 94.5 CV; Wet: 17.5 final stress, 79.2 CV; **Figure S3.2**). NMDS axes for the dry meadow described 63.7 % of the total variation in species abundances, moist meadow axes described 71.6 % variation in total, and wet meadow axes described 76.6 % variation in total (**Figure S3.2**). However, total input of N was not correlated with any axes among the different plant communities ($R^2 < 0.1$) and therefore is unlikely to be a primary driver of variation in community composition after three years of fertilization. For changes in cover of dominant species within each community I found that dry meadow dominant *C. rupestris* increased with inputs of N (**Figure 3.2A**; **Table 3.2)**, while *K. myosuroides* did not change ($p = 0.19$; **Table 3.2**). In the moist meadow neither species was affected by N inputs (*D. caespitosa*: $p = 0.09$, *G. rossii*: $p = 0.13$; **Table 3.2**). In the wet meadow I found that the cover of dominant species *C. leptosepala* didn't change with inputs of N ($p = 0.82$; **Table 3.2**), while *S. procumbens* increased with N inputs ($p = 0.002$; **Table 3.2**; **Figure 3.2B**). Species diversity, as measured using the Shannon Diversity Index (H'), increased with total inputs of N in dry and moist communities (Dry: $p = 0.01$; Moist: $p = 0.003$; **Figure 3.3; Table 3.1**), but not the wet meadow ($p = 0.64$; **Table 3.1**). Vascular plant richness did not vary with total inputs of N for any of the communities (Dry: $p = 0.21$; Moist: $p = 0.69$; Wet: $p = 0.55$; **Table 3.1**).

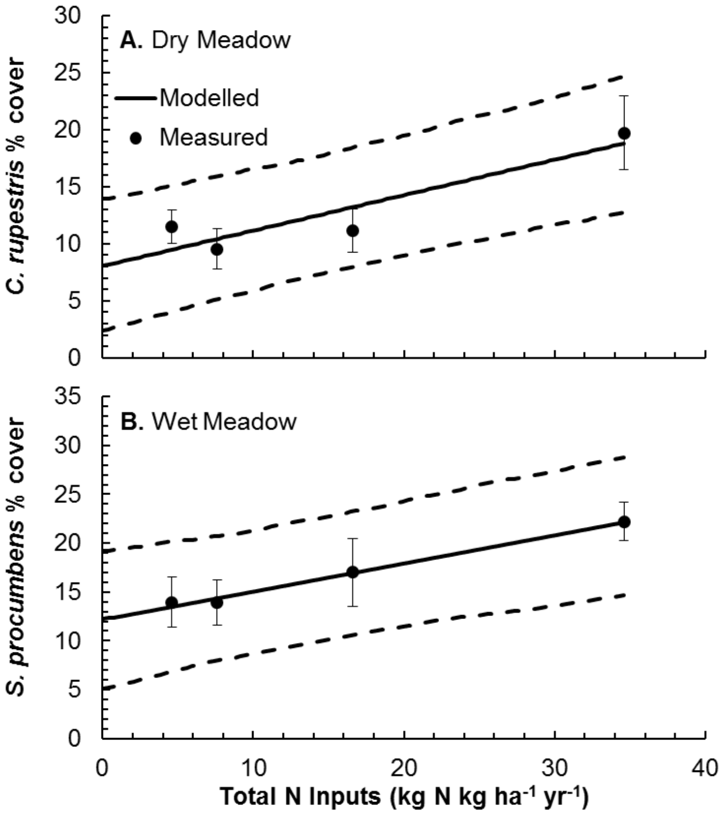

Figure 3.2. Response of dominant plant species cover to increased total N inputs for **A**. *C. rupestris*, and **B**. *S. procumbens* (2013-2015). Points represent measured treatment means ± 1 SE, while the black line indicates modelled values from the linear mixed effects model including a 95% confidence interval (CI) outlined with the dashed black line.

For my soil pools of N response metrics among communities I found that the total input of N caused an increase in resin extractable soil N in the dry meadow ($p < 0.001$; **Figure**

3.4a;**Table 3.1**), moist meadow (p = 0.01; **Figure 3.4b**), and the wet meadow community (p = 0.001; **Figure 3.4c**; **Table 3.1**). Soil pore water [NO$_3^-$], however, was not affected by increased inputs of N among all three communities (Dry: p = 0.72; Moist: p = 0.72; Wet: p = 0.44; **Table 3.1**).

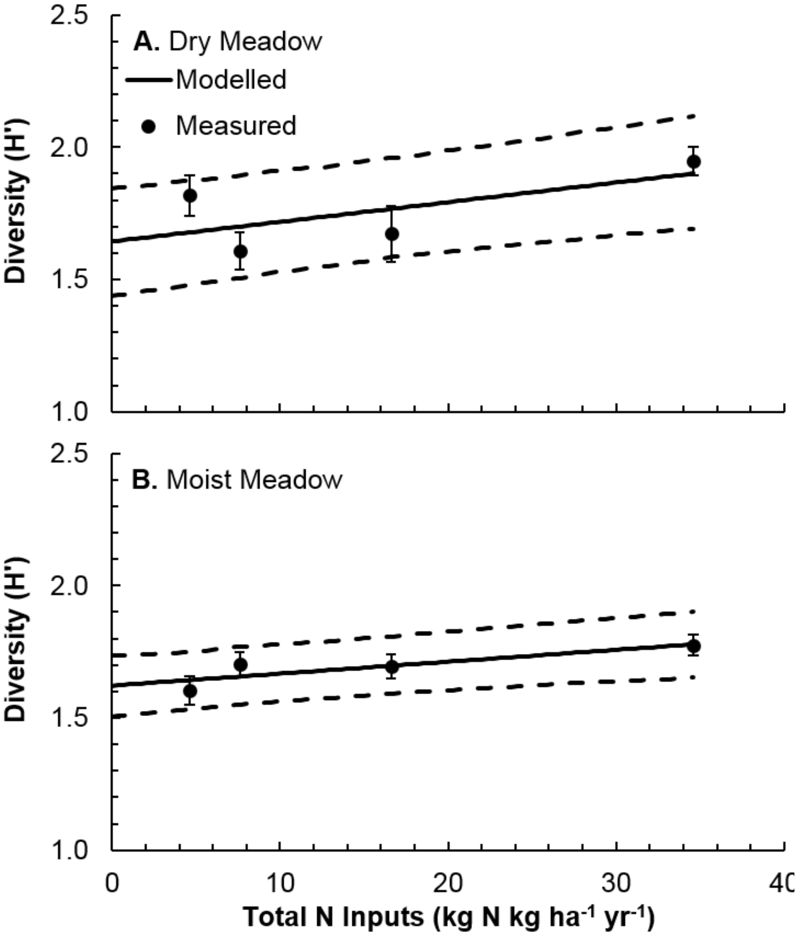

Figure 3.3. Change in species diversity in response to increased inputs of N for **A.** dry meadow communities and **B.** moist meadow communities from 2013-2015. Points represent measured treatment means ± 1 SE, while the black line indicates modelled values from the linear mixed effects model including a 95% confidence interval (CI) outlined with the dashed black line.

Ecological thresholds for plant and soil response metrics

Ecological thresholds (ET), defined as the level of N input where significant ecosystem changes occur, were calculated based on non-overlapping 95% confidence intervals between model intercept estimates (N inputs are zero) and modelled predictions of each metric responding to the range of total N input values measured in this study (**Figure 3.1**). These calculations are similar to critical loads used in air quality standards (Porter and Johnson 2007) and allow comparisons of susceptibility to change across communities and ecosystems. Relationships of each plant and soil metric response to inputs of N are shown in

Table S3.2 for models associated with changes in physiological responses and species specific evenness response metrics, and **Table S3.3** for species evenness, species losses or gains, and soil N pool response metrics. I calculated ETs for metrics that responded either positively or negatively to inputs of N (a non-zero effect size), which included four metrics for the dry meadow community, three metrics for the moist community, and two metrics for the wet community.

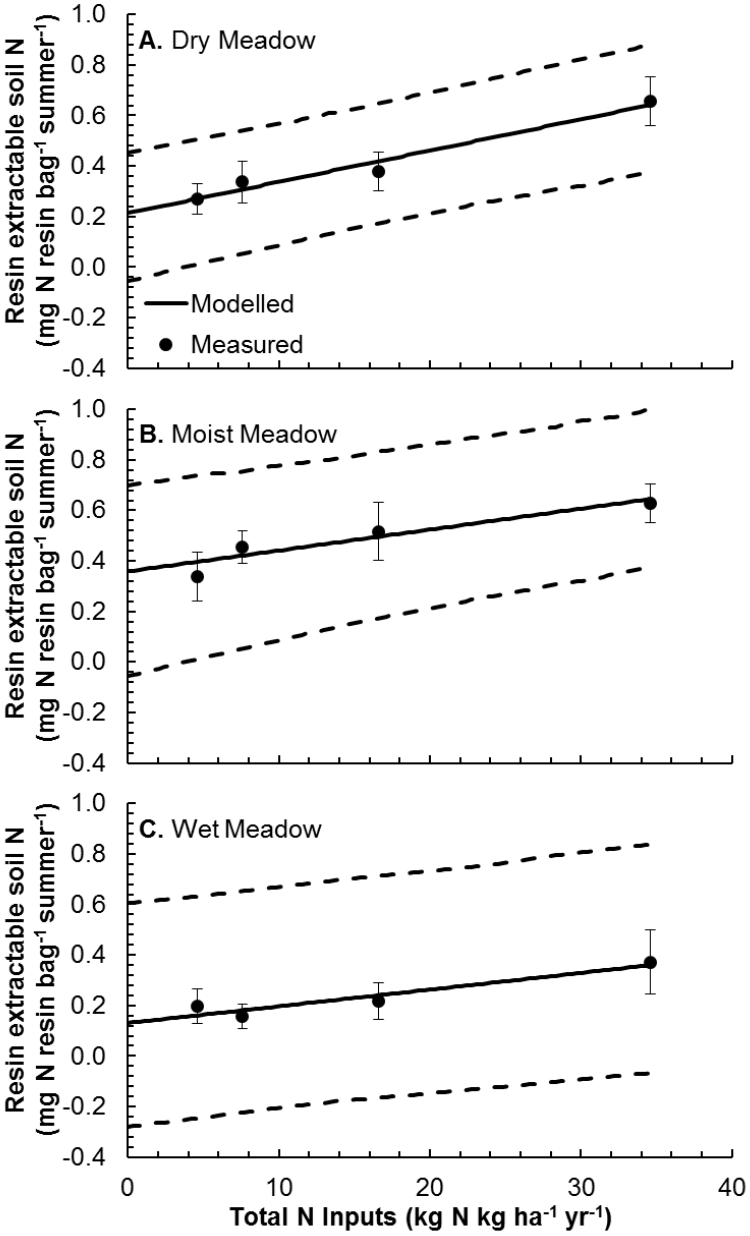

Figure 3.4. Change in resin extractable soil N associated with increasing inputs of N for **A**. dry, **B**. moist, and **C**. wet meadow communities (measured in 2013, 2015). Points represent measured treatment means ± 1 SE, while the black line indicates modelled values from the linear mixed effects model including a 95% confidence interval (CI) outlined with the dashed black line.

Physiological response metrics with ecological thresholds included the dry meadow species *C. rupestris*, which showed increases in biomass tissue concentrations of N beyond 15 kg N ha^{-1} yr^{-1}, and in litter tissues above 20.3 kg N ha^{-1} yr^{-1}. Moist meadow community species *D. caespitosa* significantly increased litter tissue concentrations of N with increased N inputs, however my ET estimate was determined as input levels greater than 34.6 kg N ha^{-1} yr^{-1} (above my experimental range of N inputs). In contrast, *G. rossii* increased in litter concentrations of N beyond 23.4 kg N ha^{-1} yr^{-1}. Neither wet meadow species responded to increased N inputs with changes in tissue chemistry. Plant responses associated with shifts in abundance for community dominant species included a significant increase in cover for the dry meadow species *C. rupestris*, and a reduction in moist meadow species *D. caespitosa*, although the ETs associated with these metrics were greater than my experimental range of N inputs. Changes in resin extractable N in the dry meadow communities occurred above 32.2 kg N ha^{-1} yr^{-1} and above 9.0 kg N ha^{-1} yr^{-1} in wet meadows communities, while the moist meadow resin extractable soil N pool did not respond to N inputs.

Discussion

The Rocky Mountain alpine contains a diverse assemblage of spatially explicit plant communities experiencing the same macro-climate (Komarkova and Webber 1978, Komarkova 1980). By implementing a N fertilization study among three alpine plant communities with varying potential biotic feedbacks and environmental conditions, I assessed how those relative differences affect a suite of plant and soil responses to elevated N deposition. This included calculating ecological thresholds associated with total inputs of N necessary to induce ecological change and how these thresholds varied among the three communities in my study. I found that increased N inputs generally affected physiological plant response metrics both more frequently and more strongly than evenness, species change, or soil response metrics, supporting previous work proposing a hierarchical response framework associated with ecosystem change in the alpine (Churchill et al. in review). Among the communities I compared, I found the strongest effect of increases in N inputs for the dry meadow, followed by the moist meadow and finally the wet meadow community. The dry meadow had the lowest average thresholds for responses to N inputs relative to the other two communities.

I was interested in two metrics of physiologically based plant responses, operating at the community scale, which may influence ecosystem responses to increased N deposition. These included the potential for increased net primary production (NPP), a commonly measured ecosystem response to global change factors across numerous ecosystems (Bowman et al. 2006, Smith et al. 2009, Isbell et al. 2015, Humbert et al. 2016), and changes in tissue concentrations of N ([N]) both for growing aboveground plant biomass and aboveground litter following fall senescence (Hobbie 1992, 2015). I expected to either see an increase in growth or an increase in foliar [N] rather than both metrics respond to increased N inputs, and predicted that communities with high ambient environmental resources such as the moist or It meadow that favored variation in plant uptake of N, would respond the most strongly to elevated N in one of these two metrics. My prediction was partially supported by the BNPP results where the wet meadow community increased belowground growth in response to increased N. These findings concur with measurements of belowground biomass among alpine communities under ambient conditions elsewhere in the Rocky Mountains, where wet meadow communities had greater growth during mid and late periods of the summer in comparison with moist and dry meadow at Niwot Ridge in Colorado (Fisk et al. 1998). On the other hand, for aboveground plant production I found that none of the three compared communities in my study responded to elevated inputs of N. In the alpine, morphological and physiological constraints on growth are known for a variety of species, including dominant species present I included in my study (Bowman and Bilbrough 2001, Meloche and Diggle 2001, Korner 2003, Bowman et al. 2006, 2012). For dry meadow communities previous studies have only found increased production following a shift in community composition favoring increased abundance of the sedge *C. rupestris* (Bowman et al. 2006, 2012). Complete compositional shifts in the dominance of this sedge and increases in grass biomass have only been observed under fertilization studies applying N at rates much higher than my highest treatment (Bowman et al. 1993, 2006, Farrer et al. 2015, Gasarch and Seastedt 2015), although changes in cover of *C. rupestris* can occur with inputs as low as 4 kg N ha^{-1} yr^{-1} (Bowman et al. 2012). Although I had predicted changes in ANPP for the moist and wet communities, I found no response in aboveground growth to increased N inputs. This lack of response may be associated with pre-formation of vegetative buds for some species (Forbis and Diggle 2001) or a co-limitation with phosphorus for others (Bowman 1994, Bowman et al. 1995). The dominant grass species in moist meadow communities has been previously shown to respond to increased N availability with increased growth (Bowman and Bilbrough 2001, Farrer and Suding 2016). However, studies focused on the growth response of this grass to N inputs, and a recent study focused on moist meadow communities along an ambient gradient of N deposition also found no relationship between N inputs and ANPP (Churchill et al. in review).

In contrast to potential morphological and physiological limitations preventing increased growth with additions of N, I did find effects of increased total N inputs on tissue [N], both for samples collected at peak biomass and for samples collected following senescence among the three communities. My observation that litter [N] was more commonly affected than living biomass [N] has important implications for plant-soil feedbacks controlling rates of decomposition, N cycling and ultimately N loss from the alpine ecosystem as N rich litter is more easily processed by soil microbes (Hobbie 1992, 2015). Additionally, increased plant foliar [N] under conditions of higher N inputs was measured in at least one dominant species for all three communities, with differing effect sizes. My results suggest that foliar [N] may be a key plant-soil feedback response metric for communities experiencing elevated N deposition, and that the strength of that feedback will be community specific associated with responsiveness traits of dominant species in each community. Studies conducted in the Rocky Mountain Ponderosa Pine forest and in deciduous forests in southern Appalachia have found foliar [N] to be strongly correlated with total inputs of N (Baron et al. 2000, Boggs et al. 2005), signifying that this response metric is both regionally important as well as applicable across multiple biomes facing similar effects of anthropogenic N deposition. Syntheses summarizing the ecological consequences of N deposition have suggested that ecosystems where plant species are unable to take up additions of N into the production of new tissues are the most susceptible to ecological changes (Matson et al. 2002, Bobbink et al. 2010). These findings further emphasize the importance of measuring physiological plant response metrics under elevated N deposition conditions.

Plant diversity is known to have important effects on N cycling within ecosystems, and thus changes in diversity in response to N deposition has the potential to contribute to cascading ecosystem change (Chapin et al. 1997, Loreau et al. 2008). In my study increased total N inputs led to increased diversity in both the dry and moist meadow communities, associated with an adjustment of evenness as sub-dominant species increased in abundance under elevated N (Bowman et al. 2006, 2012). As these sub-dominants are more responsive to N inputs, these slight changes have important implications for N cycling within the community as a whole. Numerous studies have found changes in diversity with increased N either as a decline associated with increases in abundance for already dominant species, or an increase in diversity as subdominant species become more abundant (Bobbink et al. 1998). My results contribute to a growing body of literature suggesting a non-linear relationship between inputs of N into an ecosystem and species diversity with an initial period of increased diversity until a threshold level of accumulated N facilitates competitive exclusion of rare species (Bowman et al. 2006, 2015, Simkin et al. 2016). Beyond that threshold, consistent research has shown diversity declines with increasing N deposition (Stevens et al. 2004, 2010a, Bobbink et al. 2010, Humbert et al. 2016, Simkin et al. 2016), as soil acidification associated with leaching of nitrate is generally predicted to be a key stress factor reducing community diversity (Bobbink et al. 1998, Bowman et al. 2008, Lovett and Goodale 2011).

In addition to plant uptake of N into aboveground tissues and shifts in community structure I observed an increase in resin extractable soil N with higher N inputs for all three alpine communities, although the strength of the relationship varied. I initially predicted that communities with rapid cycling between plant and soil pools associated with N deposition would similarly promote increased availability of soil N (Hobbie 2015), except in communities with high rates of N loss through leaching or denitrification. While my predicted order of community responses was not upheld, my underlying hypothesis linking plant and soil processing of N under elevated N deposition was supported (Bowman 2000). Dry meadow communities, with the strongest physiological and species evenness plant responses to N inputs, also showed the greatest increase in resin extractable soil N suggesting within growing season transfer of N between plant and soil N pools. The moist meadow showed an intermediate response in resin extractable N to deposition, while the wet meadow community, as originally predicted, showed the least change from control conditions. For the wet meadow community, high water availability in the early spring contributes to denitrification, leading these communities to act as hotspots of N processing for mobile forms of N in the alpine (Darrouzet-Nardi and Bowman 2011). Indeed, I found few plant or soil responses to N inputs in the wet meadow with notable exceptions that may be associated with changes in N pools later in the growing season. My hypothesis for these findings is that accumulated additions of inorganic N denitrifies over winter and early spring during the primary period of plant N uptake (Bilbrough et al. 2000). While early summer N additions may therefore be effectively removed from the community, and thereby prevent many plant and soil responses to elevated N, higher soil moisture later in the summer allows increased N mobility in these areas relative to the comparatively arid dry meadows (Walker et al. 1994). Mid-season additions of N then facilitate increased soil N availability in the wet meadow community, and produce both higher soil resin-extractable total N at the end of the growing season (shown in the ecological threshold estimate discussed below) and increased belowground mid and late season growth (Fisk et al. 1998).

Ecological thresholds among communities

In general, dry meadows were the most affected by N deposition, with the most plant and soil responses indicating ecological thresholds within the range of my fertilization treatments and at the lowest levels of N inputs. Physiological plant responses associated with tissue concentrations of N were the most common ecological threshold I measured, and were lowest in the dry meadow community where one of the dominant species increased foliar [N] both at peak biomass and in litter, intermediate in the moist meadow community where both dominant species had higher litter [N] relative to controls, and highest in the wet meadow community where neither species showed a response to N inputs. In contrast, wet meadow communities had the lowest ecological threshold calculated (9 kg N ha^{-1} yr^{-1}) associated with a change in soil processing of N for resin extractable soil N. One explanation for this low threshold is the mobility of soil N in this community later in the summer associated with greater soil moisture and increased microbial processing of N that may increase inorganic pools of N during this time (Walker et al. 1994, Fisk et al. 1998). As resin bags allow an integrated estimate of available N in soil through the growing season this result suggests that the timing of N deposition inputs, for example in snow pack or during mid-summer monsoons, will have important implications for wet meadow community changes.

Based on the ecological thresholds calculated for this study the critical loads, or the amount of N added below which there are no negative ecosystem consequences (Porter and Johnson 2007), associated with additions of N deposition in the alpine of the Fraser Experimental Forest were 15 kg N ha^{-1} yr^{-1} for changes in plant chemistry (not commonly reported by studies), greater than 35 kg N ha^{-1} yr^{-1} for changes in individual species cover, and 9 kg N ha^{-1} yr^{-1} for resin extractable soil N. These critical loads are higher than previous estimations of critical loads in alpine communities in the southern Rocky Mountains (species changes: 4 kg N ha^{-1} yr^{-1}-Niwot, 3 kg N ha^{-1} yr^{-1} Rocky Mountain National Park) and comparable for soil responses despite differences in resource availability among the communities included in calculations (soil N: 20 kg N ha^{-1} yr^{-1}- Niwot, 9 kg N ha^{-1} yr^{-1}- Rocky). Previous studies aimed at establishing critical loads for alpine areas in the southern Rocky Mountains have been conducted primarily in regions that have experienced a longer history of chronic N deposition (Baron 2006, Bowman et al. 2006, 2012). As a consequence, my estimates associated with ecological thresholds, especially for changes associated with plant metrics, are somewhat higher. This may be associated with the relative stage of ecosystem saturation experienced by the alpine among these different regions, and highlights the importance of sampling in areas of low ambient N deposition (Churchill et al. in review, Aber et al. 1998).

Conclusion

There is a recognized need for data supporting the establishment of ecosystem critical loads for N deposition across North America (Williams and Baron 1996, Baron et al. 2000, 2014, Porter et al. 2005, Groffman et al. 2006, Pardo et al. 2011, Simkin et al. 2016). Federal scientists in particular are foremost in identifying potential threats via N deposition to natural areas, especially in the inter-mountain west (Weathers et al. 2006, Porter and Johnson 2007, Beem et al. 2010, Benedict et al. 2013a, 2013b, Clow et al. 2015). Experimental studies aimed at establishing these ecological thresholds using fertilization have provided us with general guidelines for critical loads (Baron 2006, Bowman et al. 2006, 2012), however specific mechanisms responsible for these ecosystem responses have previously been under-examined. My present study used a comparative approach among three alpine meadow communities abundant across the southern Rocky Mountains to focus on potential differences in plant-soil feedbacks and environmental conditions as a means of examining differences in ecosystem responses. I found that not all communities responded the same way to inputs of N, and that these differences were driven by divergence in the response of specific plant and soil metrics to N deposition. Dry meadow communities had the strongest response to elevated N deposition of the three alpine communities compared for all plant and soil metrics I examined. Additionally, this community had the lowest average ecological threshold associated with responses to low dose increases in N deposition, suggesting that this community is the most susceptible to gradual ecosystem change. As dry meadow communities comprise over large percent of alpine cover, my findings have important implications for the future of alpine in the Southern Rocky Mountains, including areas protected by the Clean Air Act of the United States such as Rocky Mountain National Park. More generally, however, the diverse responses among three communities experiencing similar macro-environmental conditions suggest that plant and soil responses to N deposition can promote community specific feedbacks associated with ecosystem change.

CHAPTER 4
Variation in the stabilization of N among ecosystem pools within three alpine plant communities under the influence of N deposition

Abstract

Few studies have examined ecosystem factors that drive variation in nitrogen (N) uptake and stabilization between N pools among ecosystems, despite increased anthropogenic production of reactive N that has resulted in elevated inputs of N deposition in many areas around the globe. The consequences for long-term ecosystem responses to elevated N deposition are linked with different processes of N stabilization, or uptake, that contribute to the storage of N among ecosystem pools. Plant communities can elicit unique assemblages of potential plant-soil feedbacks contributing to N stabilization, making comparisons among communities useful in examining factors influencing N stabilization. To examine community contributions to ecosystem N stabilization I used an enriched ^{15}N isotope tracer to compare N pools associated with stabilization of N in control and N fertilization plots (30 kg N ha^{-1} yr^{-1}) among three alpine plant communities (dry, moist, and wet meadows). I found strong evidence for differences in the processes affecting N stabilization at the community scale that may have important implications for long-term ecosystem responses to N deposition. Specifically, I found significant differences in the responsiveness, or enrichment, of plant based N pools to take up ^{15}N tracer among pools within communities. The total uptake, or recovery, also varied among the three alpine communities in aboveground, belowground and soil pools of N. Total ^{15}N tracer recovery across all pools was highest in the wet meadow community, intermediate in the dry meadow, and lowest in the moist meadow community. Nitrogen addition increased the enrichment of both aboveground and belowground pools for all communities, and had a positive effect on the recovery of ^{15}N for aboveground litter among all communities and recovery of ^{15}N belowground

biomass in the dry meadow community. Total recovery among all communities remained low (6-15%), however little change in recovery of tracer between years suggests that this site has not yet reached N saturation, even under the fertilization treatment.

Introduction

Human alteration of the nitrogen (N) cycle has resulted in a drastic change in availability of biologically active N in both human-dominated and natural landscapes (Steffen et al. 2015). Increases in N-containing emissions, associated with combustion of fossil fuels and cattle feed lots, has enhanced N deposition rates globally at levels three times higher than pre-industrial values (Galloway et al. 2008). There are many ecosystem consequences associated with increased N deposition, ranging from watershed scale changes in soil chemistry and acidification, to local scale changes in plants diversity (Bobbink et al. 1998, Fenn et al. 2003a, Bowman et al. 2014). These ecosystem consequences are driven in part by whether deposited N becomes "stabilized", i.e. incorporated into plant or soil pools, or instead is lost through leaching or gaseous emissions thereby affecting other ecosystems (**Figure 4.1**) (Magill et al. 1997, Templer et al. 2012, Castellano et al. 2012, Goodale et al. 2015). Nitrogen is stabilized when taken up by plants for growth (**Figure 4.1,** Plant uptake), for example, or physically bound onto the surfaces of soil particles (**Figure 4.1,** Soil immobilization), and these processes prevent additions of N through deposition from immediately cascading into surrounding ecosystems through leaching or gaseous outputs (**Figure 4.1,** No stabilization). How the N is stabilized, where in the ecosystem it is stabilized, and how long the N remains stabilized all contribute to the net ecosystem consequences from increased N deposition, yet there are few studies examining the within ecosystem factors contributing to these processes (Templer et al. 2012).

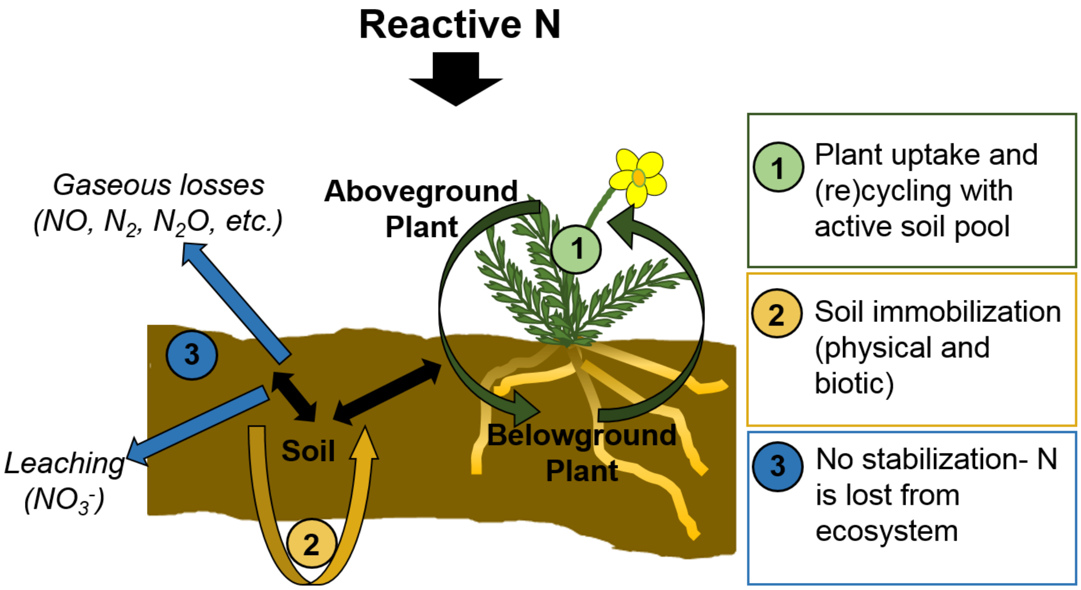

Figure 4.1. Conceptual comparison of the main categories of processes that influence uptake of N within an ecosystem. Different processes (plant uptake, soil immobilization, and no uptake) have varying impacts on the consequences of increased reactive N into an ecosystem, and may interact to differing degrees of relative importance among different community types. Pools of N influencing differences in uptake among communities are indicated in bold text, losses of N are indicated by italic text and transfer of N among mechanisms of uptake by black arrows.

Stabilized N from N deposition has important consequences for change in ecosystems, and empirical studies have frequently found increases in aboveground net primary production (Fenn et al. 2003a, Bobbink et al. 2010, Stevens et al. 2015, Humbert et al. 2016). Other responses include reductions in species richness associated with changes in species interactions in response to new resource availability (Stevens et al. 2004, Duprè et al. 2010, Harpole et al. 2016, Simkin et al. 2016). For soils, increases in N deposition often alters the microbial community, and therefore potential for soil N transformations (Nemergut et al. 2008, Ramirez et al. 2010, 2012). Furthermore, these responses often interact associated with plant-soil feedbacks that may amplify the observed ecosystem responses to increased N deposition (Hobbie 1992, 2015, Chapin et al. 1997). In comparison, N that is not initially stabilized may promote less of an immediate ecological effect locally, however, at larger spatial scales the deposited N cascades into other ecosystems. For example, the loss of N through denitrification as opposed to stabilization can result in a net reduction in locally available N and thereby reduce ecosystem consequences associated with N deposition, however this process also prompts the production of greenhouse gases (Filippa et al. 2009, Clement et al. 2012). Additionally, nitrification of deposited N promotes N loss through leaching over time, which in turn promotes soil acidification and reduces the buffering capacity of soils (Aber et al. 1998, Bowman et al. 2008).

Given the importance of N stabilization in predicting or preventing ecosystem degradation with elevated N deposition, previous research has focused primarily in understanding the partitioning of N deposition among ecosystem N pools (Buchmann et al. 1996, Bedard-Haughn et al. 2003, Friedrich et al. 2011, Choudhary et al. 2016). In general, feedbacks between plants and soils drive N stabilization in ecosystems, although direct retention of N by soils has been observed in a variety of forest systems and microbial immobilization is important especially over short time intervals (Currie et al. 1999, Zogg et al. 2000, Perakis and Hedin 2001). Soil pools of N containing recalcitrant fractions of organic components are often more stable (Neff et al. 2002) and are frequently found to be a long-term sink for N following additions (Aber et al. 1998, Fahey et al. 2011, Friedrich et al. 2011, Calvo-Fernández et al. 2015). As the duration of N being stabilized within an ecosystem is linked to the specific ecosystem pool where N becomes incorporated, the magnitude and rate of ecosystem change in response to N deposition is related to the contributions of different pools to ecosystem N stabilization (Aber et al. 1998, Currie et al. 1999).

Despite numerous studies following the fate of N within a single ecosystem, few studies have explicitly compared differences in stabilization of N in plant and soil pools among different communities. Due to the importance of the initial uptake of N by plants following N deposition, it is likely that plant community composition may play a fundamental role in determining ecosystem stabilization of N (Chapin et al. 1997). Species-level variation in N uptake occurs both through the amount of N and chemical form of N uptake from the soil and this variation likely contributes to differences in net community stabilization of N deposition (Hobbie 1992, Suding et al. 2004, Ashton et al. 2008). Similarly, higher species diversity promotes both increased responsiveness, or changes in plant uptake of N when soil availability of N changes, as well as the potential for greater N stabilization (Loreau et al. 2008). Indeed, differences in dominant plant species within a community have been found to drive changes in N stabilization among N pools (Nadelhoffer et al. 1995, Koopmans et al. 1996, Templer et al. 2005, Eickenscheidt et al. 2011). Community specific fluxes of N between plant and soil pools also act as feedbacks affecting stabilization and ecosystem changes associated with N deposition. For example, initial uptake of N into aboveground or belowground plant pools can also increase N cycling facilitated by changes in tissue chemistry favoring increased rates of decomposition, ultimately promoting increased loss of N as the ecosystem becomes more saturated in N (Aber et al. 1989, Perakis and Hedin 2001, Castellano et al. 2012, Hobbie 2015).

Due to the diverse topography within a small geographic and climatic range, alpine ecosystems contain a variety of diverse plant communities known to respond differently to N deposition, which may provide contrasting processes contributing to N stabilization in plant and soil pools (Churchill and Bowman n.d.). Additionally, high elevation areas receive higher rates of N deposition than surrounding low-lying areas, making the alpine a unique ecosystem to examine variation in N stabilization associated with N deposition. In the Rocky Mountain alpine communities including dry, moist and wet meadows contain species with traits responsive to changes in N availability (*Carex, rupestris*: Churchill and Bowman, n.d.; *Deschampsia caespitosa:* Bowman and Bilbrough, 2001; Miller and Bowman, 2003) and those that are not

(*Geum rossii*: Ashton et al., 2008). Moreover, species diversity varies among these communities (dry = wet > moist meadows) thereby increasing the potential for presence of responsive subdominant species under ecosystem change (Churchill and Bowman n.d., Walker et al. 1993, Gasarch and Seastedt 2015). In addition to potential differences in the responsiveness of plant pools to inputs of N, differences in the biomass differ (aboveground net primary production: moist> wet> dry meadows; Bowman et al., 1993; Churchill and Bowman, n.d.; Fisk et al., 1998; Gasarch and Seastedt, 2015; Walker et al., 1994). Belowground pools follow a different pattern (BNPP: wet > moist > dry meadows; Churchill and Bowman, n.d.; Fisk et al., 1998). In addition to differences in plant responsiveness and potential stabilization N inputs among different pools, there are important differences in soil among these meadows, including differences in texture (more clay in dry meadows) and soil organic matter (also higher in dry meadows) and soil moisture throughout the growing season (Walker et al. 1993, Miller and Bowman 2003). These factors all have important implications on the relative importance of N cycling through inorganic N pools, incorporation into soil organic matter complexes or immobilization in microbial biomass (Fisk et al. 1998, Neff et al. 2002).

To better understand the underlying processes associated with stabilizing the inputs of N deposition in the alpine of the Colorado Rocky Mountains, I applied enriched ^{15}N as a tracer for N uptake and multi-year N stabilization within three main pools of N. My research addressed the following questions: 1) Are there differences in the responsiveness of plant and soil N pools among alpine communities, and how does this affect N stabilization? and 2) how do additions of N affect the responsiveness and stabilization of N in plant and soil N pools within individual alpine communities as well as among the communities? I hypothesized that differences in the responsiveness of dominant species to N inputs would drive differences in enrichment of plant and soil pools among communities (Churchill and Bowman n.d., Steltzer and Bowman 1998, Bowman and Bilbrough 2001, Bowman et al. 2004). On the other hand, differences in plant composition and standing pools of N in biomass both above and belowground would produce differential multi-year stabilization of N among communities (Churchill and Bowman n.d., Walker et al. 1993, 1994, Fisk et al. 1998, Gasarch and Seastedt 2015). I predicted that differences in resource availability and species composition among alpine communities would favor increased responsiveness for aboveground plant pools in dry and moist meadow communities, and belowground plant pools in wet meadow communities (Churchill and Bowman n.d.). Due to increased N cycling between aboveground and belowground pools with soil, I additionally predicted that communities with high plant uptake of N will have lower recovery of ^{15}N tracer over annual timescales. Finally, I expected that additions of N will accelerate N cycling among pools, leading to increased losses of N and lower ecosystem stabilization of tracer N.

Methods

Field site

I addressed my research questions using a field-based fertilization experiment operating since 2013 in the alpine of the Fool Creek watershed at the Fraser Experimental Forest (Fraser), in Arapaho National Forest, Fraser, Colorado (Churchill and Bowman, in review). On-site collection of meteorological data including precipitation and temperature are maintained by Forest Service scientists at Fraser within 0.5 km from the field site location (Fool Creek meteorological station, initiated 2011). Annual average precipitation at the Fraser field station headquarters is 860 mm (elevation 2750 m), with an annual temperature maximum of 5 °C, and an annual temperature minimum of -8°C [1981-2011; Kelly Elder, personal communication].

Soils at this site are derived from granitoid parent material, and soil texture and baseline chemistry are described previously (Churchill and Bowman, in review; Retzer, 1962). The three alpine meadow communities I focused on assessing for differences in N responsiveness and stabilization were dry, moist and wet meadow communities, which are three well-studied plant communities common to the alpine of the southern Rocky Mountains (Churchill and Bowman, in review; Fisk et al., 1998; Gasarch and Seastedt, 2015; Walker et al., 2001).

I examined two levels of N addition treatments to address whether changes in total N inputs, associated with elevated N deposition in alpine areas, may influence the responsiveness and stabilization of N among these three communities. My N treatments included control plots receiving ambient deposition (4.6 kg N ha^{-1} yr^{-1}; 5 plots in each community), and a + N treatment with total N inputs of 34.6 kg N ha^{-1} yr^{-1} (ambient deposition plus 30 kg N ha^{-1} yr^{-1} fertilization; 5 plots in each community). Control and + N treatment plots were paired within a blocked design to account for spatial heterogeneity within each community, and plots were 1 m by 1.5 m in area (Churchill and Bowman, in review). Nitrogen additions for the +N treatment plots were applied as NH_4NO_3 in solution at three times during the growing season, with half the annual amount added in early spring associated with snow melt, and the remaining half divided between applications at peak biomass and post plant senescence (Bowman et al., 2012; Churchill and Bowman, in review). Control plots received the same amount of water with no dissolved N. All water used in the experiment originates from a first order alpine stream < 0.5km from the different meadow communities.

Application of enriched ^{15}N as a tracer

I applied a single dose of the isotopic tracer 98% $^{15}N\text{-}NH_4^+$ $^{15}N\text{-}NO_3^-$ (Cambridge Isotopes) following snow melt in June 2014. The tracer was applied at a rate of 0.2 kg N ha^{-1} yr^{-1}, low enough to avoid a fertilization effect in control plots while allowing us to track the movement of N into soils and plants among years and different communities. Changes in the enrichment of each pool, as well as the recovery of tracer in that pool, enabled us to quantify the responsiveness of each pool to changes in N as well as the stabilization of N. The tracer application was added to the downslope half of all plots (0.5 m by 1.5 m area) to avoid potential subsurface leaching of added enriched N (plots split lengthwise). The upslope portion of each plot served as my reference subplot for collecting natural abundance measurements of $\delta^{15}N$ within each N pool. The tracer was applied using a handheld pressure sprayer (Calvo-Fernández et al. 2015), 24 hours following the application of the spring N addition to treatment plots. Following the application of the tracer, I rinsed aboveground vegetation with 1 L/ m^2 to prevent direct uptake of labeled N by aboveground vegetation surfaces (Friedrich et al. 2011).

Field collections and laboratory processing

To address my research questions, I measured the enrichment and recovery of the isotope tracer ^{15}N through multiple ecosystem pools including aboveground vascular plant litter, belowground vascular biomass and bulk soil. I sampled these pools at two times following the tracer application, the first after one growing season (post plant senescence fall 2014) and again one year later (post plant senescence fall 2015). Paired samples of each pool were collected from reference and enriched subplots as described below and processed while spatially separated in the laboratory to avoid cross-contamination of the isotopic tracer with natural isotopic abundance.

I conducted aboveground litter harvests following plant senescence in the fall of 2014 and 2015 to determine vascular litter ^{15}N enrichment and recovery of N. These harvests included clipping a 20 cm by 20 cm section to the soil surface within the reference and enriched subplots for all control and + N treatment plots among the three alpine meadow communities (n = 120). Plant litter was initially air dried before being sorted to remove previous years' litter, lichen, cryptograms, and mosses. Vascular litter components from samples were oven dried for 48 hours at 60 °C and homogenized using liquid N and a mortar and pestle prior to sub-setting for ^{15}N elemental analysis.

I collected spatial replicates of soil cores within the reference and tracer subplots (3 cores in each subplot) for belowground biomass and bulk soil samples. Soil cores were approximately 4 cm by 4 cm, to a total depth of 15 cm- although this depth varied by community as limited by rooting zone depth associated with rock abundance. Cores within a subplot were combined in the field, and frozen until belowground biomass and soil separation. Soil cores were then thawed at room temperature and air dried prior to belowground biomass removal and soil sieving. I processed each subsample composite core using a 2 mm sieve and manually removed roots and belowground stems associated with vascular plants. These belowground biomass components were rinsed with water to remove soil particles and oven dried for 48 hours at 60 °C. Each belowground biomass sample therefore included fine and coarse roots as well as living belowground stems. I excluded decomposing roots or stems from these samples. Dried belowground biomass samples were homogenized using the same methodology as aboveground litter. I sieved remaining composite soils to remove components > 2 mm, and I dried all soil samples for 48 hours at 105 ° C. I homogenized all reference soil samples using a ball mill (Cianflone Scientific Instruments Corporation) while enriched soil samples were homogenized using a coffee grinder (Cuisinart) continuously for 30 seconds to prevent contamination between samples from reference and tracer subplots.

All samples were analyzed for $^{15}N/^{14}N$ content using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer at the Stable Isotope Facility at the University of California Davis. Plant samples were analyzed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Soil samples were analyzed using an Elementar Vario EL Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced with the same isotope ratio mass spectrometer. Replicate subsamples were submitted for analysis for approximately every eight submitted samples, for a total of 12% among all samples analyzed.

Data isotope calculations and statistical analyses

Quantifying the responsiveness to changes in N and stabilization of N in ecosystem pools of N requires measuring ratios of ^{14}N to ^{15}N in samples for a series of standardized comparisons, and I followed established procedures associated with the use of enriched ^{15}N as an ecosystem tracer for N (Nadelhoffer et al. 1995, Bedard-Haughn et al. 2003, Friedrich et al. 2011). Due to the small shifts in overall N isotopic composition among ecosystem N pools (aboveground vascular plant litter, belowground plant biomass, and bulk soil) I report shifts in ^{15}N abundance from the atmospheric standard in δ notation, such that positive values are enriched in ^{15}N relative to the atmosphere (Bedard-Haughn et al. 2003). Briefly, the ^{15}N present in samples collected from enriched and reference subplots are the ^{15}N abundance and ^{15}N natural abundance, and are determined using the δ notation of $\delta^{15}N = [(R_{sample}/R_{standard})-1]/1000$, where R_{sample} and $R_{standard}$ are the ratios between ^{15}N and ^{14}N of the sample and the standard (Buchmann et al. 1996). The

^{15}N enrichment of a labeled sample is expressed in per mil ($\delta^{15}N_{sample}$) as compared to a non-labeled sample ($\delta^{15}N_{ref}$) and in my study I use this value to express the responsiveness of a particular ecosystem N pool to take up ^{15}N. The percent of ^{15}N recovered in an ecosystem N pool (aboveground litter, belowground biomass, or bulk soil) from the total amount added is the percent stabilization of the N added via the tracer. This stabilization is determined based on the % atom excess over the atmospheric standard relative to the size of the pool and the amount of ^{15}N tracer applied in a given area (Nadelhoffer et al. 2004, Friedrich et al. 2011, Calvo-Fernández et al. 2015).

To compare differences in the enrichment (responsiveness of a pool) and recovery of $\delta^{15}N$ in different pools (amount of N stabilized) among communities and N treatment levels, I applied a linear mixed effect model in the program R using the statistical package nlme (Pinheiro et al. 2014, R Core Team 2015). For these models, community and treatment were included as interacting fixed effects. Block was included as a random effect to account for spatial heterogeneity within a community type, and year was included as a random effect to account for repeated samples within a plot over time. I applied an analysis of variance (ANOVA) test to compare whether community and treatment promoted differences in both response variables, and I used Tukey’s Honestly Significant Differences post hoc test to determine which communities were different from each other. As there were strong annual differences in both $\delta^{15}N$ enrichment and recovery between years, I additionally examined year as a fixed effect interacting with treatment for all ecosystem pools within each community type. These models included block as a random effect. For analyzing potential drivers of variation in the percent loss of enrichment in different ecosystem pools between 2014 and 2015, I created linear mixed effect models including community and treatment as interacting fixed effects, and block as a random effect. I then applied an ANOVA and Tukey HSD post hoc test to determine differences in % loss of enrichment among communities and treatment levels. All analyses were conducted using R version 3.2.3.

A number of response variables were log transformed to meet assumptions of normality associated with the mixed effects models, including: enrichment in aboveground litter, belowground biomass and bulk soil for across community comparisons, percent recovery in belowground biomass for within community comparisons, and percent recovery for aboveground litter, belowground biomass, and bulk soil for among community comparisons.

Results

Ecosystem N pools from subplots receiving the ^{15}N tracer all showed enrichment relative to reference subplots, while reference natural abundance subplots showed no evidence of cross-contamination or lateral transfer of tracer N (**Table S4.1**). Using both reference and tracer sample δ^{15}N values and the associated atom excess of ^{15}N to ^{14}N in my samples above atmospheric values, I examined the enrichment, percent recovery and percent loss of enrichment over time for three ecosystem N pools (aboveground litter, belowground biomass, and bulk soil) among three alpine communities (dry, moist, and wet meadows). My first research question examined whether there were differences in tracer enrichment and N stabilization among alpine plant communities. My second question addressed how ecosystem pools and community responses to the tracer varied between two levels of N inputs (ambient N deposition: control, and 30 kg N ha^{-1} yr^{-1}: + N treatment).

Comparison of enrichment and stabilization of N among communities

In general, aboveground litter had the greatest enrichment above δ^{15}N natural abundance

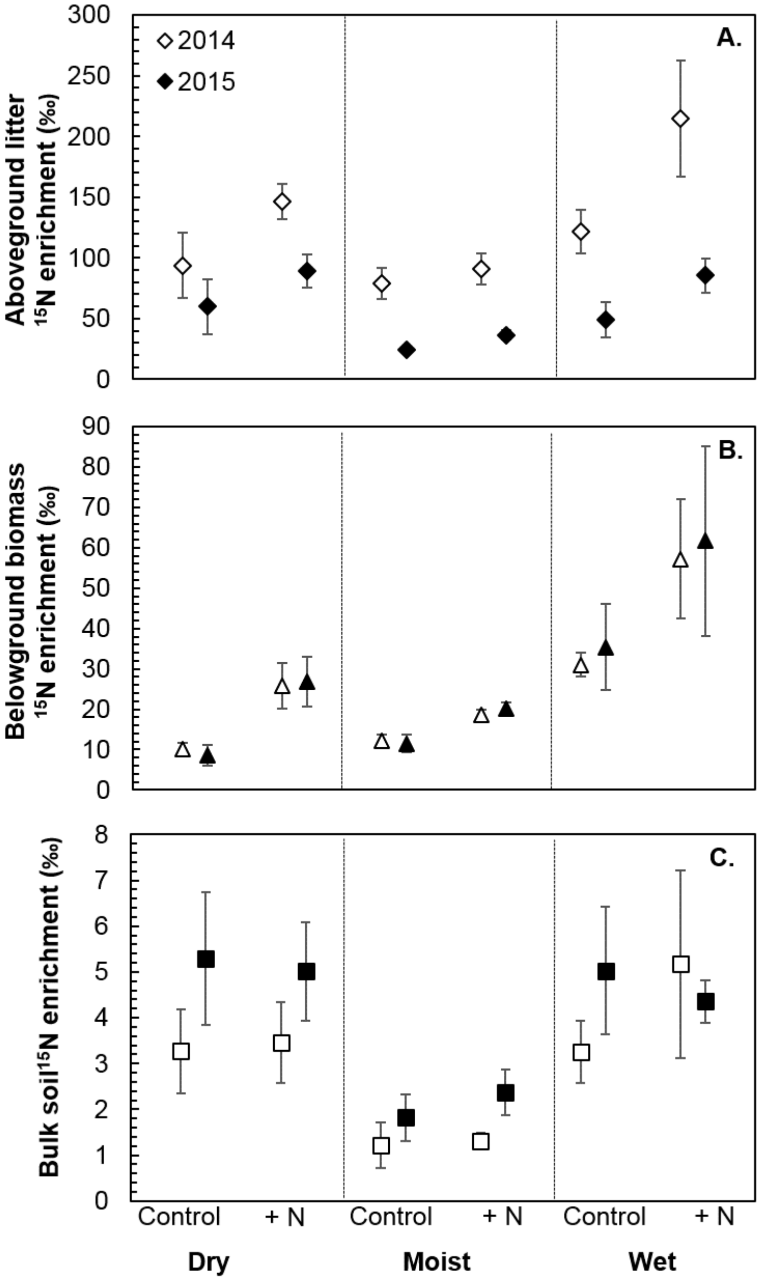

Figure 4.2. ^{15}N enrichment (‰) for samples collected in 2014 and 2015 among dry, moist and wet meadow communities with control and N addition (+ N) treatments, showing means ± 1 standard error. A) Shows vascular aboveground (AG) litter pools where there were no significant differences in enrichment among alpine communities, however the + N treatment was significantly more enriched than the control for all three communities, and all AG litter pools were less enriched in 2015 than 2014. B) Vascular belowground (BG) biomass, where the wet meadow community had significantly higher enrichment than dry and moist communities, and the + N treatment produced significantly higher enrichment in BG biomass for all communities. There were no changes in enrichment between 2014 and 2015. C) Bulk soil for the moist meadow community was significantly less enriched than either wet or dry communities, and additions of N did not significantly affect the enrichment of bulk soil. The moist meadow enrichment did increase between 2014 and 2015.

for all three alpine communities associated with the tracer application (**Figure 4.2A**), making this pool the most responsive to changes in N inputs to the ecosystem. Belowground biomass had intermediate enrichment levels (**Figure 4.2B**), and bulk soil had the least change in $\delta^{15}N$ (**Figure 4.2C**). In addressing my first research question, I found that for aboveground litter there were no differences in enrichment associated with community type (**Table 4.1**), although there was a trend for wet and dry meadows to be more enriched than moist meadow communities (**Figure 4.2A**). For belowground biomass I found that community had a significant effect on enrichment (**Table 4.1**), with wet meadow communities more enriched as compared to both dry meadow and moist meadow communities (**Figure 4.2B**). The enrichment of bulk soil was also significantly affected by community type (**Table 4.1**), such that the moist meadow was less enriched than either the dry meadow or the wet meadow community (**Figure 4.2C**). These findings suggest that the wet meadow was the most responsive to the tracer application, followed closely by the dry meadow with the moist meadow community showing the least changes in N pool enrichment from the tracer in general.

Despite high enrichment of ^{15}N in aboveground litter and moderate enrichment of ^{15}N in belowground biomass, the small overall size of these pools and multiple pathways of N loss resulted in a fairly small total recovery of the tracer ^{15}N (**Table S4.2; Figure 4.3**). I found greatest recovery of the tracer addition in the bulk soil pool, followed by the aboveground litter pool and finally the least recovery in belowground biomass (**Figure 4.3**). There was a significant difference in total recovery among communities (**Table 4.1**), where more of the tracer addition was recovered in the wet meadow than the moist meadow community (**Figure 4.3A**).

The recovery, or stabilization, of added ^{15}N tracer among the different ecosystem N pools was also affected by community type (**Figure 4.3**). Recovery of ^{15}N tracer in aboveground litter

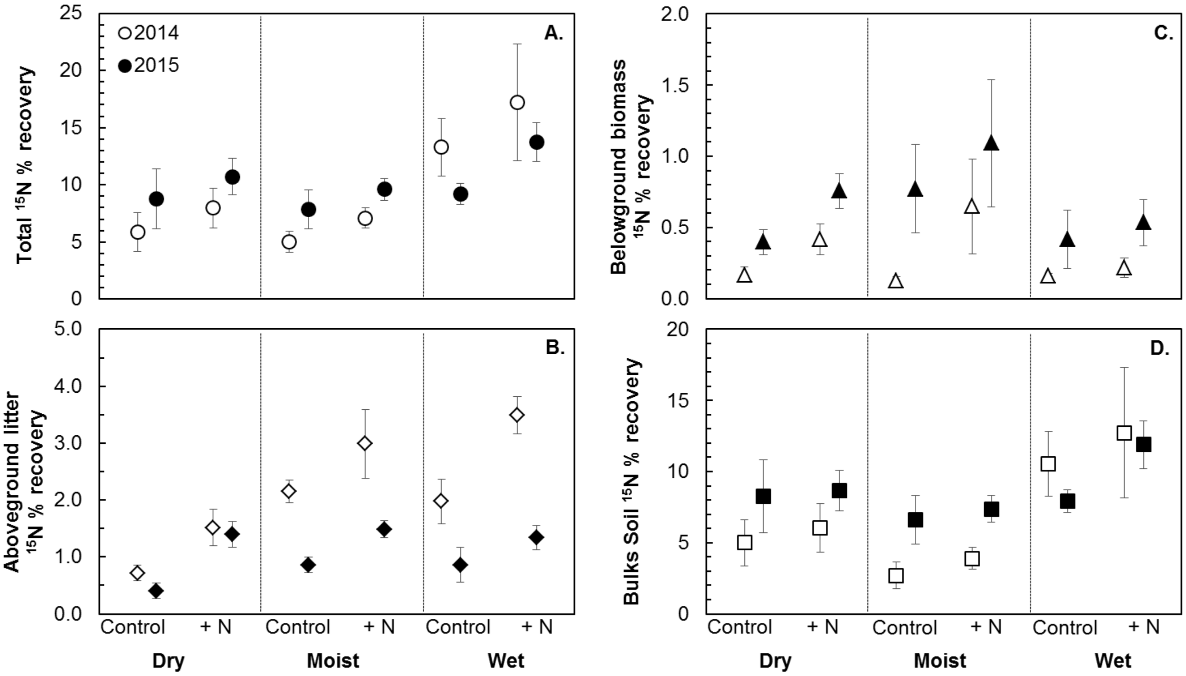

Figure 4.3. Percent (%) recovery for samples collected in 2014 and 2015 among dry, moist and wet meadow communities with control and N addition (+ N) treatments where values shown are means ± 1 standard error. A) The total recovery of ^{15}N tracer was significantly higher in the wet meadow community than the moist, although the moist meadow had increased total recovery between 2014 and 2015, and there was no significant within-community effect of + N treatment on recovery. B) Recovery in vascular aboveground (AG) litter pools was significantly lower for dry meadow than moist and wet meadow communities, and recovery was significantly increased by N fertilization for all communities. AG recovery was lower in 2015 than 2014 in moist and wet meadow communities. C) Vascular belowground (BG) biomass was significantly higher for dry than wet meadow communities and additions of N increased recovery in the dry meadow. BG biomass recovery increased between 2014 and 2015 for the moist meadow. D) Recovery in bulk soil was significantly higher in wet meadow communities than moist meadows, and recovery increased between 2014 and 2015 in the moist meadow. However, soil recovery was unaffected by N fertilization.

was significantly different among alpine communities (**Table 4.1**), with less recovery in the dry meadow than both the moist and wet meadow communities (**Figure 4.3B**). Recovery of ^{15}N tracer in belowground biomass was also significantly different among communities (**Table 4.1**), with higher recovery in dry meadow than the wet community (**Figure 4.3C**). Finally, recovery of the ^{15}N tracer in bulk soil varied among communities (**Table 4.1**), where the wet meadow was higher than the moist meadow community (**Figure 4.3D**), but not different from the dry community.

The change in the ^{15}N enrichment level between 2014 and 2015, following the single tracer application in spring 2014, was used to determine differences in community use of the tracer N over two growing seasons within each pool among my three alpine meadow communities (**Figure 4.4**). I found that in general, aboveground litter pools lost enrichment in ^{15}N between 2014 and 2015 (**Figure 4.4A**), while soils became more enriched over time (**Figure 4.4C**). Belowground biomass enrichment was more variable, and consequently didn't show a clear pattern of gains or losses in enrichment from 2014 to 2015 (**Figure 4.4B**). However, these changes in enrichment of N pools were consistent across the alpine meadow communities (**Table 4.1**).

Table 4.1. Enrichment and stabilization of N among alpine communities

Measurement	**N Pool**	**Community Effect**	**Community Comparisons**		
			Dry- Moist	***Dry-Wet***	***Moist-Wet***
Enrichment	AG Litter	$F_{2,12} = 3.71, p = 0.06$	NA	NA	NA
	BG Biomass	$F_{2,12} = 27.59, p < 0.01$	$p = 0.32$	$p < 0.01$	$p < 0.01$
	Soil	$F_{2,12} = 7.22, p = 0.01$	$p < 0.01$	$p = 0.99$	$p < 0.01$
Stabilization	Total	$F_{2,12} = 6.90, p = 0.01$	$p = 0.91$	$p = 0.13$	$p = 0.04$
	AG Litter	$F_{2,12} = 5.54, p = 0.02$	$p < 0.01$	$p = 0.01$	$p = 0.83$
	BG Biomass	$F_{2,12} = 4.43, p = 0.04$	$p = 0.17$	$p < 0.01$	$p = 0.33$
	Soil	$F_{2,12} = 5.51, p = 0.02$	$p = 0.39$	$p = 0.26$	$p = 0.01$
Δ Enrichment 2014 to 2015	AG Litter	$F_{2,20} = 3.27, p = 0.06$	NA	NA	NA
	BG Biomass	$F_{2,20} = 0.27, p = 0.76$	NA	NA	NA
	Soil	$F_{1,18} = 1.31, p = 0.29$	NA	NA	NA

Changes in enrichment and stabilization of N with elevated N inputs

My second question addressed how N pool responsiveness and recovery of the tracer label were affected by ongoing additions of N, both within and among community types between years of the study. I found a significant positive effect of + N treatment on aboveground litter enrichment (**Table 4.2**), such that plots receiving increased N fertilization were more responsive to the tracer application (**Figure 4.2A**). Additionally, all communities responded similarly, with no interaction between community type and treatment for aboveground litter enrichment. Nitrogen addition also significantly increased the relative enrichment of the belowground biomass pool (**Table 4.2**) relative to the control treatment among the three alpine communities.

On the other hand, there was no overall effect of N fertilization on bulk soil enrichment (**Table 4.2**) consistently across alpine communities.

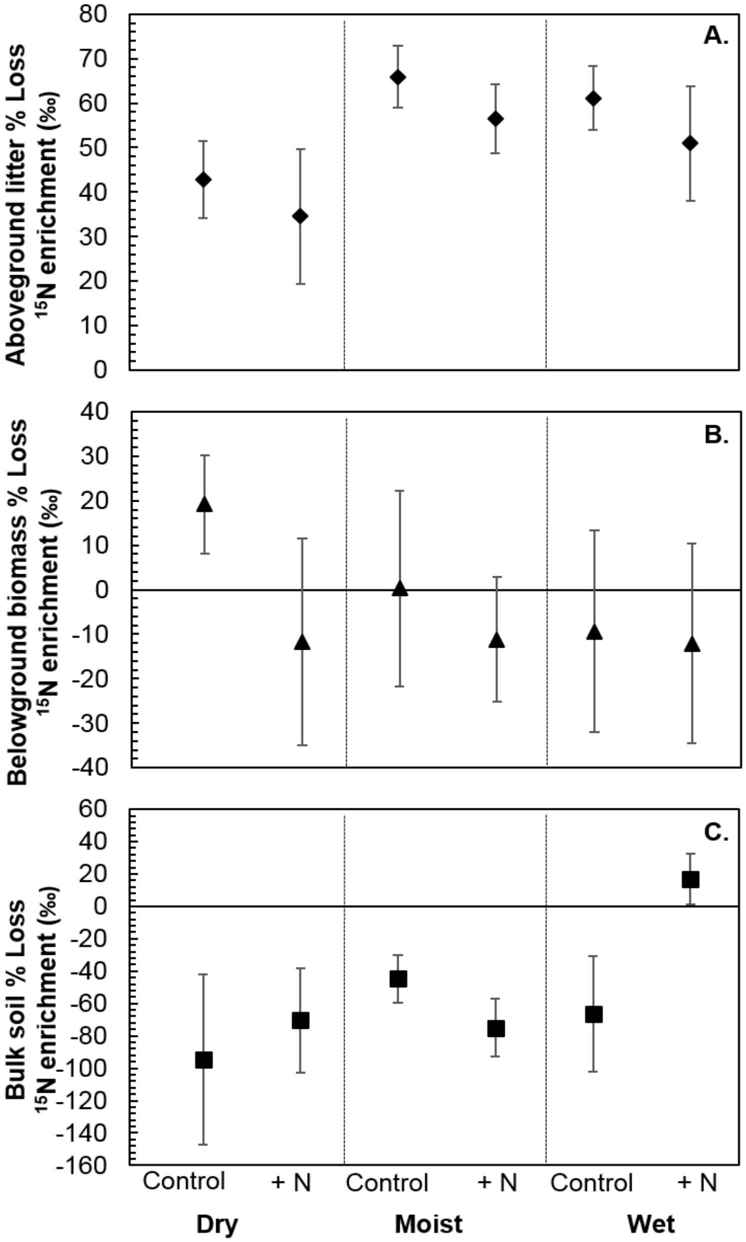

Figure 4.4. Percent enrichment lost among ecosystem N pools between samples collected in 2014 and 2015 among dry, moist and wet meadow communities with control and N addition (+ N) treatments, including A) vascular aboveground litter pools, B) vascular belowground biomass, and C) bulk soils. Values shown are means ± 1 standard error. Positive values indicate a loss of enrichment from 2014 to 2015 and negative values indicate an increase in δ^{15}N during 2015, however, there were no significant differences in the loss of enrichment among communities nor associated with N additions.

To examine how enrichment of each pool was affected by both time and N addition I analyzed each community separately. My results for the enrichment of aboveground litter showed a consistent increase with N addition within all three communities (**Figure 4.2A**). Aboveground enrichment dropped significantly from 2014 to 2015 in each community (Dry: $F_{1,12} = 5.58$, $p = 0.04$; Moist: $F_{1,12} = 66.07$, $p < 0.0001$; Wet: $F_{1,12} = 27.14$, $p = 0.0002$). Additionally, there was no effect of +N treatment on the percent loss of enrichment between years in any of the communities (Dry:

$F_{1,12} = 0.03$, $p = 0.87$; Moist: $F_{1,12} = 1.08$, $p = 0.32$; Wet: $F_{1,12} = 0.14$, $p = 0.71$). For belowground biomass I found a positive effect of +N treatment on the $\delta^{15}N$ enrichment within each community (**Figure 4.2B**), however there were no changes in enrichment of belowground biomass between years. Bulk soil enrichment was unaffected by N inputs within each community (**Table 4.2**; **Figure 4.2C**), however the moist meadow bulk soil did show an increase in enrichment between 2014 to 2015 ($F_{1,12} = 7.45$, $p = 0.02$).

Table 4.2. Effect of nitrogen fertilization on enrichment and stabilization of N among alpine communities

Measurement	**N Pool**	**Nitrogen Effect**	**Community* Nitrogen Interaction**	**Nitrogen Effect within Communities**		
				Dry	***Moist***	***Wet***
Enrichment	AG Litter	$F_{1,27} = 19.02$, $p < 0.01$	$F_{2,27} = 0.90$, $p = 0.42$	$F_{1,12} = 7.06$, $p = 0.02$	$F_{1,12} = 5.07$, $p = 0.04$	$F_{1,12} = 10.36$, $p = 0.01$
	BG Biomass	$F_{1,27} = 44.13$, $p < 0.01$	$F_{2,27} = 2.86$ $p = 0.07$	$F_{1,12} = 21.23$, $p < 0.01$	$F_{1,12} = 20.99$, $p < 0.01$	$F_{1,12} = 6.40$, $p = 0.03$
	Soil	$F_{1,27} = 1.45$, $p = 0.23$	$F_{2,27} = 0.80$, $p = 0.46$	$F_{1,12} = 0.002$, $p = 0.97$	$F_{1,12} = 1.14$, $p = 0.31$	$F_{1,12} = 0.23$, $p = 0.64$
Stabilization	Total	$F_{1,26} = 7.46$, $p = 0.01$	$F_{2,26} = 0.50$, $p = 0.61$	$F_{1,12} = 1.08$, $p = 0.32$	$F_{1,12} = 3.29$, $p = 0.09$	$F_{1,11} = 2.87$, $p = 0.12$
	AG Litter	$F_{1,27} = 28.87$, $p < 0.01$	$F_{2,27} = 2.47$, $p = 0.10$	$F_{1,12} = 23.70$, $p < 0.01$	$F_{1,12} = 4.74$, $p = 0.05$	$F_{1,12} = 10.87$, $p = 0.01$
	BG Biomass	$F_{1,27} = 11.44$, $p < 0.01$	$F_{2,27} = 1.50$ $p = 0.24$	$F_{1,12} = 10.28$, $p = 0.01$	$F_{1,12} = 3.63$, $p = 0.08$	$F_{1,12} = 1.18$, $p = 0.30$
	Soil	$F_{1,27} = 3.91$, $p = 0.06$	$F_{2,27} = 0.35$, $p = 0.71$	$F_{1,12} = 0.16$, $p = 0.70$	$F_{1,12} = 0.99$, $p = 0.34$	$F_{1,12} = 1.84$, $p = 0.20$
Δ Enrichment 2014 to 2015	AG Litter	$F_{1,20} = 1.50$, $p = 0.23$	$F_{2,20} = 0.01$, $p = 1.0$			
	BG Biomass	$F_{1,20} = 0.86$, $p = 0.36$	$F_{1,20} = 0.26$, $p = 0.77$			
	Soil	$F_{1,18} = 1.39$, $p = 0.27$	$F_{2,18} = 1.39$, $p = 0.27$			

Total stabilization, or recovery, of the tracer among combined N pools was positively affected by inputs of N (**Table 4.2**; **Figure 4.3A**) when examining across all three communities in the alpine, with communities responding similarly to the treatment. The recovery of added ^{15}N tracer among the individual ecosystem N pools was also affected by increased inputs of N (**Figure 4.3**). There was significantly greater recovery of tracer for +N treatments plots in aboveground litter for all three communities (**Table 4.2**). Additionally, for belowground biomass I found increased recovery of ^{15}N in +N treatment plots (**Figure 4.3C**), with no interaction between community and N treatment (**Table 4.2**). However, increased N inputs did not influence recovery of the isotopic tracer in bulk soil (**Figure 4.3D**).

When examining the recovery of the isotopic tracer within each community between years, I found that total recovery increased between 2014 and 2015 in the moist meadow community ($F_{1,12}$ = 6.34, p = 0.027) but not in the dry ($F_{1,12}$ = 2.09 p = 0.17) or wet meadow communities ($F_{1,12}$ = 2.56, p = 0.14). For aboveground litter tracer recovery, increased N inputs had a positive significant effect within all three individual communities (**Table 4.2**; **Figure 4.3B**), while recovery was lower in 2015 than 2014 for both the moist meadow ($F_{1,12}$ = 17.47, p = 0.001) and wet meadow communities ($F_{1,12}$ = 29.29, p = 0.0002). N stabilization in belowground biomass increased with the + N treatment for the dry meadow, but not in the moist or wet meadow community (**Table 4.2**). Recovery also increased from 2014 to 2015 in belowground biomass for the moist meadow ($F_{1,12}$ = 27.00, p = 0.0002), but there was no difference in recovery between years in the dry meadow ($F_{1,12}$ = 0.01, p = 0.91) or the wet meadow community ($F_{1,12}$ = 3.70, p = 0.08). The percent recovery of tracer from soils within each community was unaffected by inputs of N (Dry: $F_{1,12}$ = 0.03, p = 0.87; Moist: $F_{1,12}$ = 0.05, p = 0.83; Wet: $F_{1,12}$ = 0.14, p = 0.72). While the recovery of ^{15}N tracer from bulk soils did increase between 2014 to 2015 in the most meadow community ($F_{1,12}$ = 13.85, p = 0.003), this was not true in the dry ($F_{1,12}$ = 2.47, p = 0.14) or wet meadow communities ($F_{1,11}$ = 0.66, p = 0.43).

Finally, the loss of enrichment between 2014 and 2015 was unaffected by N inputs for all ecosystem N pools (**Figure 4.4**), and there was no interaction among communities and levels of N inputs (**Table 4.2**).

Discussion

While there is a rich body of literature examining variation in stabilization of N among pools across diverse ecosystems in response to changes in N availability (Templer et al. 2012), the specific factors affecting ecosystem responsiveness to changes in N and stabilization of that N are less well established (Corre et al. 2010). The responsiveness of an ecosystem to changes in N is indicative of how rapidly different pools may change to accommodate new resources, while stabilization is associated with the proportion of added N that is retained among different pools and biotic feedbacks have been suggested to play a role in these processes (Chapin et al. 1997, Loreau et al. 2008). In this study I focused on comparing three plant communities containing contrasting potential for plant responsiveness and abiotic conditions that may influence N pools and cycling. My first question examined community variation in responsiveness of ecosystem pools to a ^{15}N tracer as well as the recovery of N tracer, while my second question addressed how the influence of fertilization may alter these pools. I found that the alpine wet meadow community was consistently the most responsive to the tracer application, while the moist meadow had the least enrichment among all pools. The wet meadow also had the greatest total recovery of the ^{15}N tracer among the communities I compared. The addition of N at 30 kg N ha^{-1} yr^{-1} above ambient inputs resulted in an increase in enrichment for above and belowground plant pools for all communities, as well as an increase in the total recovery of ^{15}N tracer among all communities, particularly associated with increases in plant associated pools.

I found that total recovery of the isotopic tracer among the alpine communities was low, with only 6-11% stabilization under control conditions, although there were key differences among communities. While recovery of tracer applications across ecosystems have been measured as high as 100% in some forested ecosystems (Buchmann et al. 1996), ecosystems lacking a woody overstory are on average much lower at ~50% (Templer et al. 2012). Research conducted within montane slopes in the Rocky Mountains have also shown still lower total recovery of isotopic tracers, associated with high leaching losses (16% total recovery; Hinckley et al., 2014) and similarly high fluxes of N from the alpine may be a driving force behind the low recovery from studies examining alpine plant (6%; Bowman et al., 2006) or soil pool N stabilization such as my study. Despite a low total recovery across the alpine, I found no increased losses of ^{15}N tracer over time, suggesting that retained N is being cycled within the ecosystem between years and provides no evidence that the alpine of Fraser Experimental Forest is approaching saturation from N inputs (Aber et al. 1998, Nadelhoffer et al. 1999). This conclusion is further supported by the increase in recovery for aboveground (all communities) and belowground (dry communities) plant pools associated with elevated inputs of N with my fertilization (Templer et al. 2012).

Comparison of enrichment and stabilization of N among communities

There are many ecosystem factors that could drive variation in the responsiveness of the N pools and the stabilization of N among communities, including plant associated factors such as community specific differences in species abundance, diversity of resource use, and the amount of primary production. My initial prediction associated with community scale differences in the responsiveness and recovery of ecosystem N pools to the tracer application was conditionally supported, as I did not find differences in aboveground enrichment among communities in my study suggesting that all communities contained species responsive to spring inputs of N tracer. In the belowground biomass pool, however, the wet meadow community was the most responsive to the ^{15}N tracer, as I predicted, potentially associated with differences in the timing of new root growth in this community due to higher water availability later in the growing season

(Fisk et al. 1998). I found that recovery of the ^{15}N tracer within the aboveground plant pool did differ among communities, driven by greater aboveground plant biomass for the moist and wet meadows (**Table S4.2**; Churchill and Bowman, in prep; Fisk et al., 1998; Gasarch and Seastedt, 2015). In comparison, the dry meadow community stabilized more ^{15}N tracer in the belowground plant pool than the wet community, possibly associated with greater cover of small statue forbs than in similar alpine communities at Niwot Ridge (Bowman, 1994; Churchill and Bowman, in prep; Fisk et al., 1998; Gasarch and Seastedt, 2015; Walker et al., 1993). Several studies in natural ecosystems report high total recovery of N for ecosystems with high initial plant uptake of N and recovery (90-100%) (Nadelhoffer et al. 1995, Buchmann et al. 1996, Koopmans et al. 1996, Gurmesa et al. 2016), suggesting that the common responsiveness of alpine plant pools is an important feature likely to affect ecosystem responses under changes in N deposition. Additionally, this initial uptake in ecosystems dominated by herbs and deciduous trees can promote loss over time as litter N becomes part of the soil, thereby having cascading influence on multiple pools that affect total ecosystem responses to change in nutrient availability (Calvo-Fernández et al. 2015).

Aside from plant mediated drivers of ecosystem responsiveness and stabilization of N, there are key soil factors including texture, moisture content, pH, organic matter concentrations, as well as microbial components. These drivers have a direct effect on soil pools of N, yet despite diverse abiotic conditions among the three alpine communities I compared, I found relatively few differences in the soil pool responsiveness and stabilization of N. Indeed, among all communities, soil was the most substantial pool contributing to ecosystem total recovery of the ^{15}N tracer. These findings concur with previous studies that follow the movement of N beyond a single growing season, where soils are frequently the largest long-term pool associated with N stabilization (Nordin et al. 1998, Zogg et al. 2000, Corre et al. 2010, Friedrich et al. 2011, Goodale et al. 2015, Choudhary et al. 2016, Zajac and Blodau 2016).

In my study, however, this comparative null result is likely associated with contrasting processes of stabilization operating among the different communities I examined. For example, based on previous comparative studies examining microbial processing of nutrients among alpine communities, and measurements of differences in physical attributes between soil pools, soils in the wet community may support greater microbial stabilization than the other two communities as a result of higher soil moisture (Fisk et al. 1998). On the other hand, high soil responsiveness in the dry meadow may be due to abiotic stabilization, as there was little change in the enrichment of soil over time in this community and other studies conducted in dry meadows at other sites have shown low microbial activity and potential for immobilization due to low soil moisture (Fisk et al. 1998). This community in particular has soil containing high silt and clay content and soil organic matter, which are also important in soil immobilization of N, and suggest that long-term stabilization of N in this community may eventually surpass the wet meadow communities (Churchill and Bowman, in prep; Perakis and Hedin, 2001; Seastedt, 2001; Zogg et al., 2000). This pattern of stabilization has important consequences for future leaching under sustained N inputs, due to the high soil moisture content in wet communities in particular. Multiple mechanisms contribute to both physical and biological stabilization of N in the soil, and plant-soil feedbacks between active pools of N within bulk soil provide a clear linkage for short term differences in stabilization between plant and soil pools of N (Zogg et al. 2000, Perakis and Hedin 2001, Castellano et al. 2012).

Changes in enrichment and stabilization of N with elevated N inputs

One factor affecting the variation in responsiveness of ecosystems to changes in N as well as stabilization of N is how much N has accumulated in the ecosystem already (Aber et al. 1998, Corre et al. 2010, Humbert et al. 2016). Chronic additions of N deposition alter existing pools of N within an ecosystem, and have the potential to disrupt fluxes between plant and soil pools(Churchill et al. n.d., Smith et al. 2009, Lovett and Goodale 2011). Contrary to my initial prediction of increased loss of tracer with N addition, I found that the addition of N at a rate 7.5 times higher than current N deposition rates increased the total recovery of tracer ^{15}N relative to the control plots by 140%. Previous work synthesizing the effects of increased N inputs on ecosystem retention of a ^{15}N tracer has suggested a threshold relationship exists, where low levels of N inputs increase total recovery of tracer but high levels of N inputs reduce recovery (Templer et al. 2012). More recent studies conducted in diverse ecosystems support this finding (Choudhary et al. 2016, Gurmesa et al. 2016), although prior to my study there were no established patterns of changes in recovery with increased N inputs for alpine ecosystems (Bowman et al. 2006).

I found that alpine plots receiving N additions generally showed increased responsiveness and recovery of the ^{15}N tracer in both aboveground and belowground plant pools in a general pattern across the alpine. This occurred despite no increase in aboveground biomass with N addition (**Table 4.2**), although there was an increase in belowground primary productivity in the wet meadow (Churchill and Bowman, in prep). Instead, I found a trend for increases in the concentration of N in plant tissues with inputs of N (**Table 4.2**), which is supported by previous research at this site and throughout the southern Rocky Mountains (Churchill et al., in review; Churchill and Bowman, in prep). These results suggest that higher retention of ^{15}N tracer in plant pools associated with N additions was driven by observed changes in plant physiology and abundance associated with the fertilization over time (Bowman et al., 2012, 2006; Churchill and Bowman, in prep). In comparison, limited response of tracer recovery in the soil pool to elevated N inputs was expected, as elevated N inputs have only been occurring at this site for three years and treatment plot soils have had little total N accumulation relative to the existing pool of N (Aber et al. 1998, Fenn et al. 2003a). Consequently, plant-soil feedbacks that may affect decomposition rates and cycling of N may not yet have produced an impact on the soil N pool in this system (Hobbie 2015).

Despite a general increase in responsiveness and recovery associated with fertilization among all community types in the alpine, meadows relied on different pools of N in stabilizing increased inputs of N. Dry meadows primarily showed changes in the responsiveness of the aboveground litter N pool and recovery in the belowground plant pool. In comparison the moist meadow had evidence of aboveground and belowground transfers of N over time, and the wet meadow community retained the greatest amount of ^{15}N tracer in the soil pool despite substantial losses in aboveground recovery between years. High stabilization within a pool with rapid turnover, such as belowground plant biomass (Fisk et al. 1998), may lead to increased ecosystem change as N affects a hierarchy of ecological responses with increased N (Churchill et al., in review; Smith et al., 2009). The dry community has been previously identified as being the most susceptible to plant and soil change in response to additions of N and my findings suggest that increases in the dominance of a responsive sedge species, and shifts in evenness for subdominant species, may facilitate more rapid cycling between plant and soil pools of N (Churchill and Bowman, in prep).

Conclusion

Comparing the pools of N among alpine plant communities provided an evaluation of potential processes affecting the stabilization of N, and the potential sources of variability in the fate of N within ecosystems comprised of heterogeneous landscapes. Few studies have examined variation in N stabilization between N pools within ecosystems, and I found strong evidence for differences in the processes affecting N stabilization at the community scale that may have important implications for long-term ecosystem responses to N deposition. Furthermore, I found that while all alpine communities were more responsive to tracer inputs under fertilization conditions, differences in the stabilization of that N among plant and soil pools have different implications for long-term ecosystem responses to N deposition. While my natural communities necessarily confound many of the potential factors that drive ecosystem responsiveness to changes in N as well as N stabilization, my findings of differences in allocations of N among pools with community type suggests that, especially in heterogeneous landscapes, this variation may play an important role in N cycling.

CHAPTER 5
Conclusion

As a plant ecologist I am interested in the contributions of the plant community to changes in ecosystem processing of N associated with global change, and in my dissertation I examined these potential contributions related to ecosystem responses to N deposition. The goals of the research presented here was to examine alpine plant and soil responses to N deposition 1) in multiple spatial scales throughout the Southern Rocky Mountains, 2) among diverse plant communities associated with unique environmental conditions common in the alpine of this region, and 3) by examining potential ecosystem factors that determine N uptake and stabilization through comparing pools of N within those communities.

I conducted research to address these goals in the southern and central Rocky Mountains, among three alpine plant communities that span a soil hydrology gradient and contribute to ecosystem heterogeneity within the alpine (Walker et al. 1993, Seastedt et al. 2004). The alpine faces a number of unique challenges associated with ecosystem processing of N under elevated inputs including a short growing season that limits plant uptake of N, plant physiological constraints on growth, shallow and poorly weathered that limit accumulation of soil pools of N, and a strongly seasonal pulse of increase N availability associated with the melting of accumulated snow (Bowman 1992, 2000, Williams and Baron 1996, Meloche and Diggle 2001, Darrouzet-Nardi et al. 2012). Due to these limitations even slight differences in cycling of N between plants and soils may have important implications for variation of responses of the alpine to increased N.

In Chapter 2 I developed a response framework of expected ecosystem changes associated with increasing N inputs based on cumulative responses of plant and soil pools of N (Aber et al. 1998, Smith et al. 2009, Lovett and Goodale 2011). There are many known ecosystem consequences from increased N deposition, and ongoing research is establishing thresholds associated with how much N may be added to an ecosystem before irreversible change occurs (Clark and Tilman 2008, Gaudnik et al. 2011). Which ecosystem metrics to use for these thresholds, however, has not been thoroughly addressed and considering metrics as proposed indicators of ecosystem changes in an integrated framework (**Figure 2.1**) enables comparisons among studies using different types of metrics.

In using an ambient gradient of N deposition to test the proposed framework, I found that a number of ecosystem responses were correlated with ambient N deposition, including the tissue N concentration of dominant plant species at peak biomass, and abundance of a nitrophilic dominant grass species. These species are known to have an influence on N processing in soil, and are therefore important potential plant-soil feedback mechanisms facilitating future ecosystem responses to environmental changes. Additionally, I found that there were significant differences in species composition among sites receiving different levels of N deposition, although there was only a weak negative correlation between diversity and N deposition. I found a significant positive correlation between nitrate concentration in soil solution and N deposition during two years of sampling, while all other soil metrics showed no changes with N deposition. These findings suggest that moist meadows in the southern Rocky Mountain alpine are being affected by N deposition with minimal soil biogeochemical changes but substantial biotic impacts.

The research I present in Chapter 3 extends the response framework designed in Chapter 2 to compare ecosystem responses to N deposition among multiple plant communities. A second goal for this chapter was to examine the potential for differences in the amount of N needed to induce change among plant and soil metrics. By establishing a N fertilization experiment in three common alpine plant communities, in an ecosystem with historically minimal chronic inputs of N (Argerich et al. 2013), I was able to capture potentially early signals of ecosystem change in response to N inputs. My results supported the cumulative aspect of the Chapter 2 response framework, with more fast stage metrics changing in response to N inputs, and at lower total inputs of N, than medium or slow stage metrics. Additionally, I found that not all communities responded the same way to inputs of N, and that these differences were driven by divergence in the response of specific plant and soil metrics to N deposition. Ongoing research at Niwot Ridge supports the finding of community specific changes with increases in N and phosphorus availability (Bowman et al. 1993, 1995, Gasarch and Seastedt 2015); however my research was the first comparison of differences in the response of each community to a sequence of low dosage N inputs. Community scale processes have been shown to confer stabilizing feedbacks to environmental changes in many ecosystems, however, less experimental work has focused on making comparisons across communities (Loreau and de Mazancourt 2013). My goal was to use biotic and abiotic differences among three alpine communities as a means of examining variation in plant and soil responses that contribute to ecosystem change.

Through this comparison, I found that dry meadow communities had the strongest response to elevated N deposition of the three alpine communities examined. The dry meadow also had the lowest average ecological threshold associated with responses to low dose increases in N deposition, suggesting that this community is the most susceptible to gradual ecosystem change. This finding is in contrast to studies conducted at Niwot suggesting that in general this community is resistant to change in N availability in comparison with other alpine meadows dominated by more responsive grasses (Bowman and Bilbrough 2001, Miller and Bowman 2003). One reason for this potential difference is the relative lower accumulated N in the soils of my field site (Fraser) in comparison to ambient conditions at Niwot Ridge and especially to the experimental manipulations of N additions. Most of those studies have drawn conclusions of alpine change based on high total inputs of N, at levels over 8 times greater than my highest fertilization treatment (Bowman 1994, Bowman et al. 1995) or in coordination with other nutrient amendments (Gasarch and Seastedt 2015). However, my findings highlighting the increased potential for dry meadow plant responsiveness to N additions measured in Chapter 3 were instrumental in interpreting enrichment of plant pools with ^{15}N tracer results in Chapter 4.

Processes of N stabilization or uptake contribute to the storage of N among ecosystem pools, each with potentially different consequences for long-term ecosystem responses to elevated N deposition (Templer et al. 2012). Plant communities can illicit unique assemblages of potential plant-soil feedbacks contributing to N stabilization, making comparisons among communities useful in examining factors influencing N stabilization (Chapin et al. 1997, Hobbie 2015). Given strong community specific responses of both plants and soils in Chapter 3, I was interested in examining the fate of N among ecosystem pools of N that may contribute to ecosystem responses. To examine these differences I used an enriched ^{15}N isotope tracer in Chapter 4 to compare N pools associated with stabilization of N in control and N fertilization plots among the three alpine plant communities (dry, moist, and wet meadows). I found significant differences in the responsiveness, or enrichment, of pools to take up ^{15}N tracer among pools within communities, with high enrichment in all pools for the wet meadow community and low enrichment of all pools in the moist meadow community.

The total uptake, or recovery, also varied among the three alpine communities in aboveground, belowground and soil pools of N. Total ^{15}N recovery across all pools was highest in the wet meadow community, intermediate in the dry meadow, and lowest in the moist meadow community. Nitrogen addition increased the enrichment of both aboveground and belowground pools for all communities, and had a positive effect on the recovery of ^{15}N for aboveground litter among all communities and recovery of ^{15}N belowground biomass in the dry meadow community. Total recovery among all communities remained low (6-15%), however little change in recovery of tracer between years suggests that this site has not yet reached N saturation, even under the fertilization treatment.

Comparing the pools of N among alpine plant communities provided an evaluation of potential processes affecting the stabilization of N, and the potential sources of variability in the fate of N within ecosystems comprised of heterogeneous landscapes. Few studies have examined variation in N stabilization between N pools within ecosystems, and I found strong evidence for differences in the processes affecting N stabilization at the community scale that may have important implications for long-term ecosystem responses to N deposition.

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APPENDIX CHAPTER II

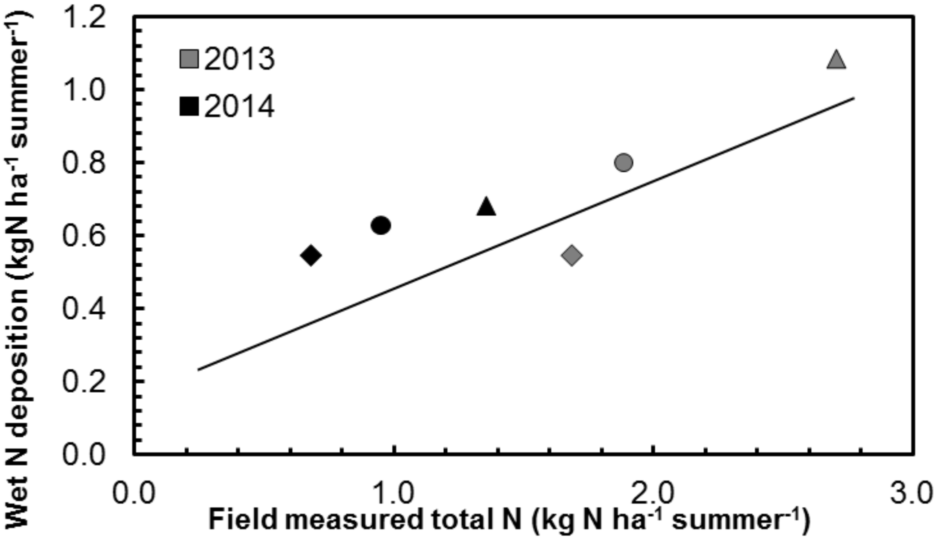

Figure S2.1 Field measured bulk deposition of N at Niwot Ridge (triangles), Rocky (circles), Fraser (diamonds) between 2013- 2014 during summer months as compared to measurements of wet N deposition during the same periods of time collected near each field site.

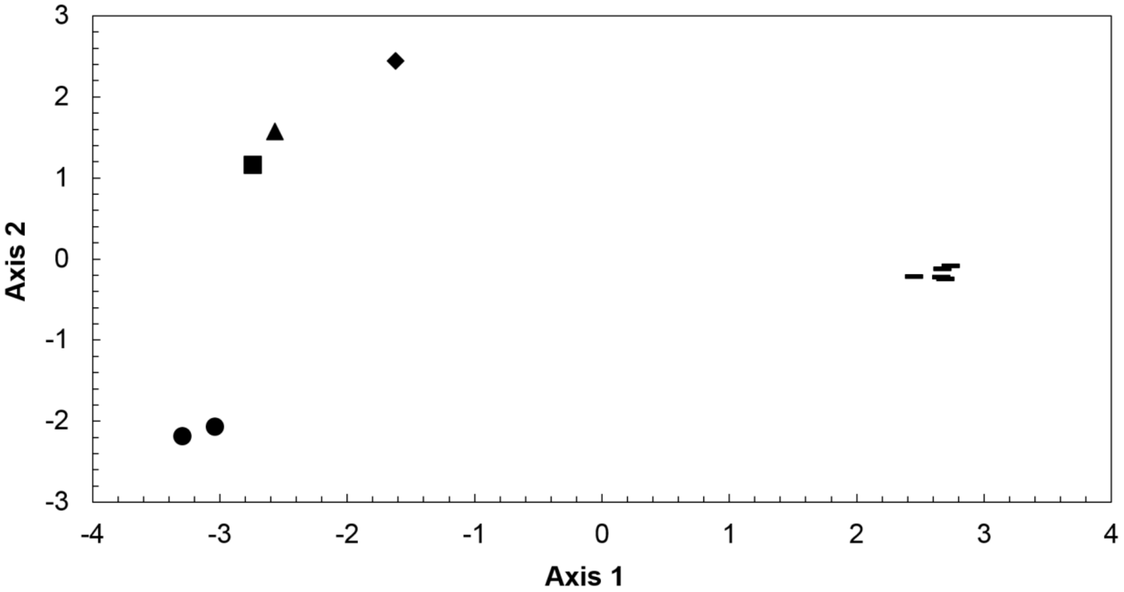

Figure S2.2. Principle Components Analysis ordination showing axis 1 (71.8% variation) and axis 2 (19.1 % variation) of bedrock oxides for rock samples collected at each site. Arapaho is shown as a diamond, Fraser as a square, Niwot as a triangle, ROMO as a circle, and Shoshone as a dash.

Table S2.1 Bedrock and soil chemistry associated with each site along the nitrogen deposition gradient

Element		Fraser	Niwot	Rocky	Shoshone	Torreys
Al	*Oxide*	16.0	13.9	14.5	14.5	15.0
	Cation	1.69 ± 0.26*	2.32 ± 0.29	0.15 ± 0.01	0.15 ± 0.01	4.00 ± 0.29
Ca	*Oxide*	1.1	0.6	6.2	6.2	1.3
	Cation	9.39 ± 0.77	6.19 ± 0.64	16.59 ± 0.99	16.59 ± 0.99	4.04 ± 0.49
Fe	*Oxide*	2.8	2.0	6.1	6.1	3.9
	Cation	0.04 ± 0.01	0.18 ± 0.03	0.05 ± 0.01	0.05 ± 0.01	0.07 ± 0.01
K	*Oxide*	5.1	5.1	2.7	2.7	6.3
	Cation	0.50 ± 0.04	0.41 ± 0.05	0.75 ± 0.04	0.75 ± 0.04	0.36 ± 0.02
Mg	*Oxide*	0.7	0.7	6.5	6.5	0.8
	Cation	1.67± 0.15	1.06 ± 0.11	3.99 ± 0.23	3.99 ± 0.23	0.73 ± 0.08
Mn	*Oxide*	0.0	0.0	0.1	0.1	0.0
	Cation	0.30 ± 0.05	0.11 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.29 ± 0.04
Na	*Oxide*	2.9	2.2	4.2	4.2	2.0
	Cation	0.09 ± 0.01	0.06 ± 0.01	0.06 ± 0.0	0.06 ± 0.0	0.05 ± 0.0

* Values shown are means ± 1 standard error for cation soil chemistry samples

Table S2.2 Ecosystem plant indicators best fit model estimates for nitrogen deposition and/or precipitation

Ecosystem indicator	**Intercept**	**N Deposition (Measured)**	**Precipitation (Average Local)**	**R^2-m***	**R^2-c$^\wedge$**
D. caespitosa ANPP	7.37 ± 2.34	0.87 ± 1.51		0.005	0.154
	6.66 ± 3.06		0.002 ± 0.003	0.007	0.154
G. rossii ANPP	5.88 ± 4.03	0.88 ± 2.82		0.007	0.505
ANPP	94.81 ± 58.1		0.113 ± 0.06	0.056	0.252
	125.52 ± 41.7	51.45 ± 29.2		0.055	0.252
D. caespitosa biomass N%	0.55 ± 0.44	1.16 ± 0.30		0.548	0.760
	-0.12 ± 0.61		0.003 ± 0.001	0.558	0.768
G. rossii biomass N%	1.13 ± 0.60	0.73 ± 0.41		0.338	0.827
	0.63 ± 0.78		0.002 ± 0.001	0.391	0.827
D. caespitosa litter N%	-0.16 ± 0.87	-2.23 ± 1.22	0.005 ± 0.003	0.287	0.781
G. rossii litter N%	1.26 ± 0.30	-0.14 ± 0.20		0.056	0.631
	1.40 ± 0.39		-0.0004± 0.0004	0.085	0.626
D. caespitosa Cover	-0.01 ± 0.35	0.40 ± 0.25		0.284	0.870
	-0.20 ± 0.51		0.0008 ± 0.0006	0.262	0.875
G. rossii Cover	0.30 ± 0.09	0.016 ± 0.06		0.001	0.203
Diversity	2.29 ± 0.0.36	-0.41 ± 0.25		0.133	0.551
	2.41 ± 0.55		-0.0008 ± 0.0006	0.107	0.564
	1.81 ± 0.80	-1.17 ± 1.13	0.0017 ± 0.0025	0.140	0.576
Richness	9.10 ± 3.33	-17.01 ± 5.43	0.032 ± 0.012	0.214	0.512

*Marginal R^2- associated with fixed effects only

$^\wedge$Conditional R^2- associated with both fixed and random effects

Table S2.3 Ecosystem soil indicators best fit model estimates for nitrogen deposition and/or precipitation

Ecosystem indicator	Intercept	N Deposition (Measured)	Precipitation (Average Annual)	R^2-m*	R^2-c^
Soil resin extractable N	0.24 ± 0.17	0.13 ± 0.07		0.062	0.635
	0.06 ± 0.29		0.0004 ± 0.0003	0.066	0.521
Soil pore water [NO_3^-]	2.08 ± 2.03		-0.0049 ± 0.0015	0.102	0.818
	2.71 ± 1.93	1.13 ± 0.71	-0.0073 ± 0.0023	0.219	0.763
Soil pH	1.75 ± 0.15	-0.19 ± 0.10		0.359	0.924
	1.81 ± 0.23		-0.0004 ± 0.0003	0.256	0.928
Soil pH (no Shoshone)	1.53 ± 0.15	-0.06 ± 0.10		0.065	0.839
Soil CEC	25.86 ± 6.46	-9.90 ±4.49		0.369	0.804
	29.47 ± 10.39		-0.0193 ± 0.0113	0.279	0.813
Soil CEC (no Shoshone)	14.24 ± 7.31	-2.64 ± 4.73		0.044	0.631
Soil C:N	10.57± 0.60	-2.41 ± 0.92	0.0057 ± 0.0020	0.130	0.312

*Marginal R^2- associated with fixed effects only

^Conditional R^2- associated with both fixed and random effects

APPENDIX CHAPTER III

Table S3.1 Environmental and biotic traits associated with different alpine meadow communities

Community	**% Clay**	**% Sand**	**pH**	**CEC**
Dry	36.0 ± 2.1	39.0 ± 5.1	5.39 ± 0.07	21.7 ± 1.4
Moist	17.0 ± 1.5	70.7 ± 3.7	4.53 ± 0.05	16.2 ± 1.1
Wet	21.3 ± 4.5	63.3 ± 5.0	4.28 ± 0.05	14.3 ± 0.6

Table S3.2 Relationship between total N input and dominant plant species indicators of ecosystem responses and ecological thresholds (ET) associated with those indicators for samples collected in year 3 of the study (2015)

Community	**Indicator**	**Intercept**	**N Addition**	**R^2-m**	**R^2-c**	**P Value**	**ET**
Dry	*C. rupestris* Biomass N%	1.55 ± 0.10	0.023 ± 0.004	0.630	0.687	<0.001	15.0
	K. myosuroides Biomass N%	2.57 ± 0.08	0.003 ± 0.004	0.040	0.211	0.34	NA
	C. rupestris Litter N%	0.99 ± 0.08	0.015 ± 0.003	0.430	0.731	<0.001	20.3
	K. myosuroides Litter N%	1.78 ± 0.09	0.002 ± 0.002	0.017	0.655	0.35	NA
	C. rupestris Cover	5.73 ± 4.02	0.383 ± 0.145	0.178	0.513	0.02	> 34.6
	K. myosuroides Cover	47.30 ± 7.79	-0.113 ± 0.262	0.005	0.480	0.67	NA
Moist	*D. caespitosa* Tissue % N	2.80 ± 0.13	-0.002 ± 0.004	0.004	0.519	0.70	NA
	G. rossii Tissue %N	2.44 ± 0.11	0.003 ± 0.006	0.024	0.072	0.55	NA
	D. caespitosa Litter N%	1.52 ± 0.10	0.007 ± 0.004	0.088	0.429	0.11	> 34.6
	G. rossii Litter N%	1.47 ± 0.09	0.013 ± 0.004	0.299	0.362	0.01	23.4
	D. caespitosa Cover	88.16 ± 4.77	-0.429 ± 0.188	0.158	0.426	0.04	> 34.6
	G. rossii Cover	29.23 ± 4.80	-0.251 ± 0.205	0.058	0.272	0.24	NA
Wet	*C. leptosepala* Tissue N%	2.30 ± 0.09	0.004 ± 0.004	0.046	0.046	0.35	NA
	S. procumbens Tissue N%	2.25 ± 0.10	0.003 ± 0.005	0.013	0.013	0.63	NA
	C. leptosepala Litter N%	1.52 ± 0.08	0.006 ± 0.004	0.099	0.191	0.15	NA
	S. procumbens Litter N%	1.93 ± 0.08	0.001 ± 0.004	0.001	0.001	0.88	NA
	C. leptosepala Cover	5.93 ± 1.86	-0.021 ± 0.081	0.003	0.203	0.80	NA
	S. procumbens Cover	11.80 ± 3.53	0.233 ± 0.146	0.090	0.332	0.13	> 34.6

[^] fourthroot transformation on resin bag N measured in mg N resin bag^{-1} 92 days^{-1}

[#] ln transformed soil pore water [NO_3^-] measured in mg N/L

Table S3.3 Relationship between total N input with plant community and soil indicators of ecosystem responses and ecological threshold (ET) associated with those indicators for data collected in year 3 (2015) of the fertilization experiment

Community	Indicator	Intercept	N Addition	R^2-m	R^2-c	P Value	ET
Dry	Diversity	1.70 ± 0.15	0.006 ± 0.005	0.035	0.437	0.29	NA
	Richness	18.93 ± 1.45	0.014 ± 0.067	0.002	0.140	0.84	NA
	Resin extractable soil N[^]	0.35 ± 0.09	0.011 ± 0.004	0.247	0.466	0.01	32.2
	Soil pore water [NO_3^-][#]	-5.42 ± 0.02	-0.02 ± 0.01	0.115	0.115	0.18	NA
Moist	Diversity	1.63 ± 0.08	0.003 ± 0.003	0.034	0.522	0.26	NA
	Richness	13.42 ± 0.65	-0.020 ± 0.024	0.022	0.412	0.41	NA
	Resin extractable soil N[^]	0.58 ± 0.07	0.004 ± 0.003	0.091	0.091	0.22	NA
	Soil pore water [NO_3^-][#]	-5.09 ± 0.55	0.017 ± 0.028	0.019	0.019	0.19	NA
Wet	Diversity	2.28 ± 0.10	-0.002 ± 0.003	0.015	0.462	0.47	NA
	Richness	18.68 ± 1.01	-0.052 ± 0.036	0.061	0.449	0.17	NA
	Resin extractable soil N[^]	0.24 ± 0.04	0.014 ± 0.002	0.738	0.738	< 0.001	9.0
	Soil pore water [NO_3^-][#]	-6.24 ± 0.12	-0.007 ± 0.006	0.061	0.061	0.28	NA

[^] fourthroot transformation on resin bag N measured in mg N resin bag^{-1} 92 days^{-1}

[#] ln transformed soil pore water [NO_3^-] measured in mg N/L

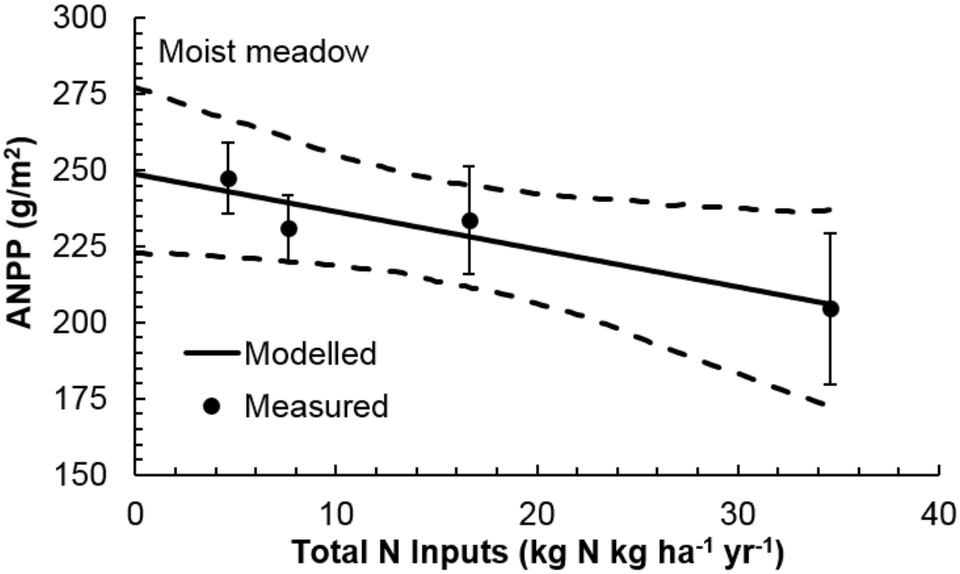

Figure S3.1 Aboveground vascular net primary production from samples collected at peak biomass during the summer of 2015 in the moist meadow community, in the third year of the study for total community ANPP

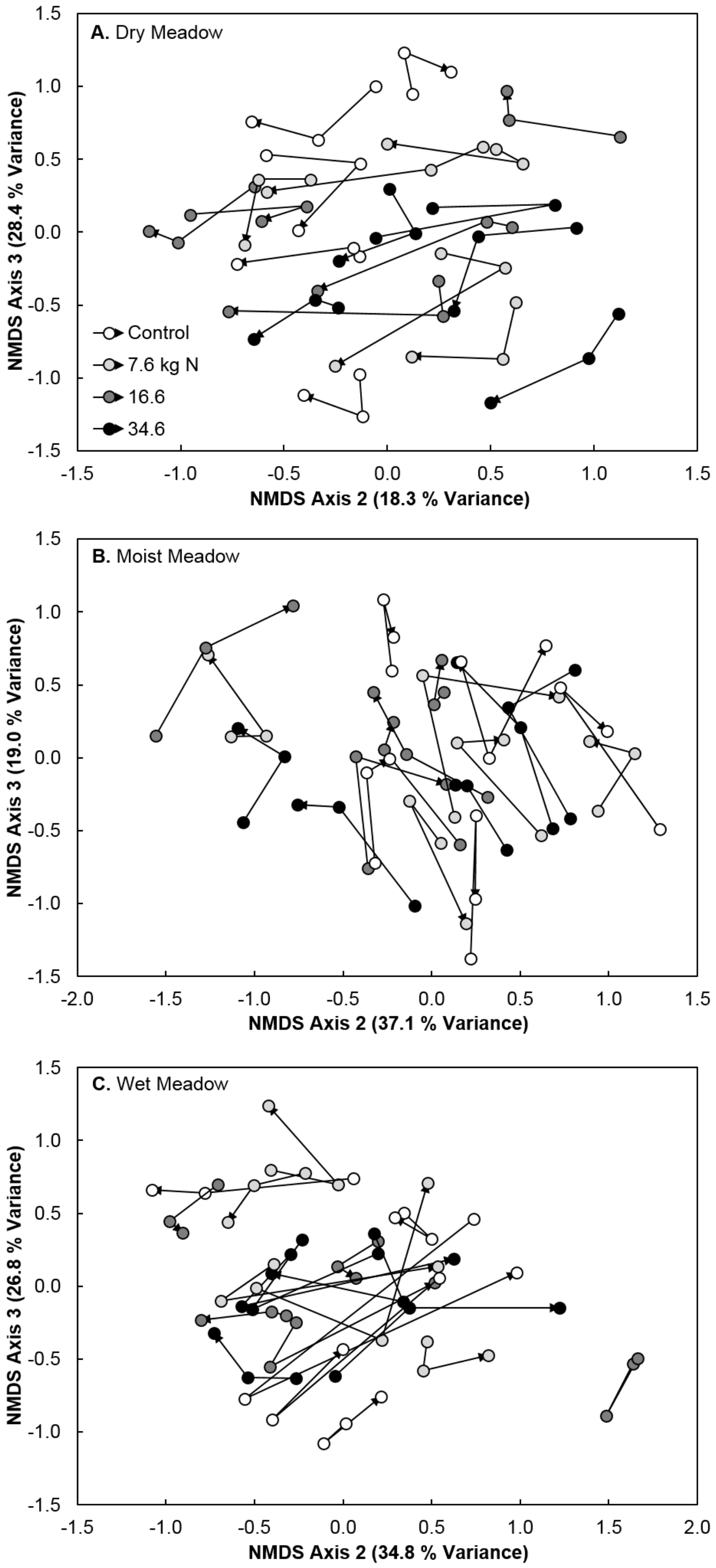

Figure S3.2 Non-metric Multi-Dimensional Scaling ordinations of plant species abundances 2013-2015 for plots responding to elevated rates of total N inputs in the **A.** dry meadow (19.9 stress), **B.** moist meadow (18.9 stress), and **C.** wet meadow (17.5 stress). Vectors within each panel indicate changes in species composition of individual plots through time, and colors indicate the total level of N inputs (kg N ha^{-1} yr^{-1}).

APPENDIX CHAPTER IV

Supplemental Table 4.1. δ^{15}N abundance in non-labeled (Ref) and ^{15}N labeled subplots among community N pools for control and N addition (+ N) treatments

Community	Year	Treatment	Above		Below		Soil	
			Ref.	**^{15}N**	**Ref.**	**^{15}N**	**Ref.**	**^{15}N**
Dry	2014	Control	-2.45 (0.22)[†]	91.06 (27.04)[‡]	0.44 (0.26)	7.56 (0.86)	4.27 (0.18)	7.55 (0.86)
		+ N	-0.17 (0.37)	146.05 (14.52)	0.61 (0.35)	7.74 (0.93)	4.27 (0.32)	7.74 (0.93)
	2015	Control	-2.41 (0.71)	57.33 (22.74)	0.12 (0.33)	8.72 (2.35)	4.11 (0.54)	9.42 (1.32)
		+ N	2.53 (1.29)	91.56 (13.47)	2.67 (0.94)	29.52 (6.09)	4.56 (0.46)	9.59 (1.47)
Moist	2014	Control	1.02 (0.55)	80.29 (12.93)	-0.04 (0.26)	-0.04 (0.26)	4.58 (0.20)	5.79 (0.39)
		+ N	4.92 (1.86)	96.52 (12.17)	0.88 (0.46)	6.00 (0.51)	4.68 (0.30)	3.00 (0.23)
	2015	Control	0.95 (0.44)	24.89 (2.52)	0.69 (0.29)	12.19 (2.43)	4.29 (0.27)	6.11 (0.33)
		+ N	3.10 (0.66)	39.72 (4.18)	1.97 (0.61)	22.03 (1.90)	4.62 (0.25)	6.99 (0.42)
Wet	2014	Control	1.44 (1.80)	123.26 (18.01)	1.40 (0.42)	8.33 (0.55)	5.05 (0.24)	8.33 (0.55)
		+ N	2.59 (2.00)	217.68 (46.04)	0.88 (0.27)	10.04 (2.00)	4.85 (0.18)	10.04 (2.01)
	2015	Control	-1.84 (0.49)	47.18 (14.22)	2.04 (0.46)	37.54 (10.21)	5.55 (0.28)	10.61 (1.35)
		+ N	1.52 (0.84)	86.99 (13.12)	1.25 (0.50)	63.05 (23.46)	4.94 (0.15)	9.32 (0.43)

[†] Values are δ^{15}N in ‰ as means and parentheses indicate one standard error

[‡] Positive values are enriched relative to the atmosphere

Supplemental Table 4.2. Size of ecosystem N pools among the three alpine meadow communities as influenced by N addition treatment and between years

Community	Year	Treatment	Above		Below		Soil	
			Mass (g/m^2)	% N	Mass (g/m^2)	% N	Mass (kg/m^2)	% N
Dry	2014	Control	68.29 (15.17)	1.64 (0.06)	260.49 (30.05)	0.73 (0.02)	30.76 (1.46)	0.69 (0.03)
		+ N	87.94 (12.19)	1.79 (0.09)	263.31 (34.55)	0.78 (0.03)	31.72 (1.55)	0.76 (0.04)
	2015	Control	75.58 (8.18)	1.66 (0.05)	356.50 (39.02)	0.82 (0.04)	34.36 (3.78)	0.70 (0.07)
		+ N	128.26 (8.90)	1.85 (0.06)	293.68 (42.89)	0.93 (0.04)	34.37 (3.08)	0.74 (0.06)
Moist	2014	Control	229.02 (26.02)	1.80 (0.06)	326.73 (145.00)	1.09 (0.06)	36.54 (2.89)	1.16 (0.08)
		+ N	205.76 (22.25)	1.95 (0.07)	232.97 (46.14)	1.05 (0.04)	40.96 (5.24)	1.09 (0.05)
	2015	Control	259.02 (18.28)	1.91 (0.06)	405.75 (80.16)	1.29 (0.08)	29.61 (5.11)	1.44 (0.09)
		+ N	262.14 (15.54)	2.17 (0.06)	328.55 (63.51)	1.39 (0.04)	32.04 (4.53)	1.18 (0.09)
Wet	2014	Control	138.29 (15.54)	1.50 (0.06)	545.71 (102.10)	1.10 (0.07)	52.98 (3.30)	0.89 (0.05)
		+ N	150.61 (22.01)	1.55 (0.05)	482.18 (80.06)	1.18 (0.08)	48.39 (4.63)	1.07 (0.04)
	2015	Control	128.88 (16.67)	1.73 (0.05)	154.35 (33.15)	1.06 (0.07)	34.23 (2.77)	0.92 (0.10)
		+ N	126.71 (11.95)	1.93 (0.06)	150.30 (22.01)	1.07 (0.06)	33.95 (3.52)	1.03 (0.05)

*Values are means and parentheses indicate one standard error