NITROGEN CRITICAL LOADS FOR ALPINE VEGETATION AND TERRESTRIAL ECOSYSTEM RESPONSE: ARE WE THERE YET?

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Abstract. Increases in the deposition of anthropogenic nitrogen (N) have been linked to several terrestrial ecological changes, including soil biogeochemistry, plant stress susceptibility, and community diversity. Recognizing the need to identify sensitive indicators of biotic response to N deposition, we empirically estimated the N critical load for changes in alpine plant community composition and compared this with the estimated critical load for soil indicators of ecological change. We also measured the degree to which alpine vegetation may serve as a sink for anthropogenic N and how much plant sequestration is related to changes in species composition. We addressed these research goals by adding 20, 40, or 60 kg N ha⁻¹ yr⁻¹, along with an ambient control (6 kg N ha⁻¹ yr⁻¹ total deposition), to a species-rich alpine dry meadow for an eight-year period. Change in plant species composition associated with the treatments occurred within three years of the initiation of the experiment and were significant at all levels of N addition. Using individual species abundance changes and ordination scores, we estimated the N critical loads (total deposition) for (1) change in individual species to be 4 kg N ha⁻¹ yr⁻¹ and (2) for overall community change to be 10 kg N ha⁻¹ yr⁻¹. In contrast, increases in NO₃⁻ leaching, soil solution inorganic NO₃⁻, and net N nitrification occurred at levels above 20 kg N ha⁻¹ yr⁻¹. Increases in total aboveground biomass were modest and transient, occurring in only one of the three years measured. Vegetative uptake of N increased significantly, primarily as a result of increasing tissue N concentrations and biomass increases in subdominant species. Aboveground vegetative uptake of N accounted for <40% of the N added. The results of this experiment indicate that changes in vegetation composition will precede detectable changes in more traditionally used soil indicators of ecosystem responses to N deposition and that changes in species composition are probably ongoing in alpine dry meadows of the Front Range of the Colorado Rocky Mountains. Feedbacks to soil N cycling associated with changes in litter quality and species composition may result in only short-term increases in vegetation N pools.

Key words: alpine; community change; diversity; mineralization; N cycling; N deposition; nitrification; Rocky Mountains.

INTRODUCTION

Deposition of anthropogenically derived reactive nitrogen (N) has increased substantially over the past half century as a result of increased agricultural, industrial, and dispersed suburban development (Galloway and Cowling 2002, Howarth et al. 2002). Although N remains a common limiting resource for plant growth in many temperate terrestrial ecosystems (Vitousek and Howarth 1991), ecological responses to increased N supply include not only increases in primary production, but also changes in diversity of both native and exotic species (Bobbink et al. 1998, Gough et al. 2000, Zavaleta et al. 2003), alteration of internal system N and C cycling, plant susceptibility to stress, and eventually changes in cation biogeochemistry and acidification of soils (Vitousek et al. 1997, Aber et al. 1998, Neff et al. 2002).

Land managers and policy makers have evaluated the impacts of pollutants on ecosystems using the “critical load” concept (e.g., Bobbink et al. 2002, Porter et al. 2005). Critical loads are generally defined as the minimum input of a pollutant that causes a significant “harmful effect” to a sensitive ecological indicator and are used by the European Union and Canada to set air pollution standards (e.g., Nilsson and Grennfelt 1988). Ecological indicators of N deposition have traditionally emphasized soil-based measures of inorganic N and N cycling as the metrics of the system response. However, changes in sensitive biota may occur before these functional changes occur and may provide an early warning prior to more harmful effects, such as loss of diversity and acidification of surface waters. Changes in species composition have been used to provide specific signals from environmental change and make sensitive environmental monitors (Philippi et al. 1998). Estimat-
ing the N critical loads for biotic change, particularly in sensitive vegetation types, is an important goal of land managers in wilderness areas and national parks in the United States (Adams 2003, Fenn et al. 2003a, Porter et al. 2005).

The sensitivity of terrestrial ecosystems to N deposition depends on rates of N deposition, as well as the sink strengths of plants, soils, and soil microorganisms for inorganic N (Boring et al. 1988, Lovett 1994, Fenn et al. 1998). In addition, the physical environment, through selection pressures on the component organisms, influences the biotic capacity to take up increasing N inputs by affecting growth rates and resource acquisition capacity (Aerts and Chapin 2000). In nutrient-poor ecosystems plants exhibit low resource uptake and tissue turnover rates, and thus the biotic constraint on plant responses to increases in external N inputs is particularly acute (Chapin et al. 1997, Bowman 2000). These criteria suggest herbaceous-dominated systems of climatically extreme environments should be highly susceptible to ecological impacts and experience ecological changes at relatively low rates of N deposition. Such ecosystems often have plant communities containing species that differ in their capacity to respond to changes in resource availability, and as a result changes in the composition of plant species may precede changes in the pattern of ecosystem responses (e.g., soil N transformations) anticipated according to theories of N saturation (Aber et al. 1998). Changes in plant diversity may therefore prove be an early indication of N saturation and changes in aquatic biogeochemistry (Lokke et al. 1996).

The goal of our research was to estimate the N critical load for vegetation (composition and abundance) and soils (soil solution inorganic N, mineralization, and nitrification) in an alpine dry meadow in the southern Rocky Mountains of Colorado, USA, adjacent to the Denver metropolitan area. High-elevation mountain ecosystems are potentially sensitive indicators of N deposition effects for several reasons. First, climatic controls on decomposition severely constrain the supply of N to plants, and as suggested above, the biotic capacity to take up and use N in growth is relatively low (Bowman and Bilbrough 2001). As a result, increases in external N supply in alpine communities typically result in changes in community diversity, with increases in the abundance of nitrophilous species (Theodose and Bowman 1997). Second, rates of N deposition are higher in high-elevation sites due to greater amounts of precipitation and associated wet deposition, particularly on the eastern side of the continental divide of the southern Rocky Mountains (Baron et al. 2000). Third, direct anthropogenic influences on ecosystem function (e.g., land use change and intensification) are minimal, and thus confounding factors that may interfere with detection of the effects of N deposition are low. Finally, soils in alpine areas tend to be thin and lightly weathered, and as a result have a low capacity to filter inorganic N (Williams et al. 1996, Williams and Tonnessen 2000; although see Liu et al. [2004] for an evaluation of surface water and ground water interactions in talus-dominated systems). Many parks and wilderness areas in the western United States are classified as Class I areas under the Clean Air Act Amendments of 1977; this status affords them special protection from the effects of air pollution (NPS ARD 2002).

**Materials and Methods**

**Study site**

The research was carried out in an alpine dry meadow at 3500 m on Niwot Ridge, Colorado, a United Nations Educational, Scientific, and Cultural Organization (UNESCO) Biosphere Reserve and a National Science Foundation-sponsored Long Term Ecological Research site. Dry meadows occupy ~40% of the alpine on Niwot Ridge (Komárková 1979) and are dominated by the sedge *Kobresia myosuroides,* with a mix of perennial forbs, sedges, and grasses making up the remainder of the community. Climate at the site is typical of a mid-latitude temperate alpine site, with long, cold winters and short, cool, sometimes dry summers (Greenland and Losleben 2001). Mean annual precipitation is ~900 mm, with a spring (March and April) maximum. The growing season commences in late May to early June and concludes in early August. Soils are Cryumbrepts, with ~25% organic matter in the surface horizons, with mineral soil derived from acidic granitic parent material (Seastedt 2001). N deposition at the site, both wet and dry, is 6 kg ha\(^{-1}\) yr\(^{-1}\), with approximately equal contributions from NH\(_3\) and NO\(_x\) (Sievering 2001).

We selected a dry meadow site for several reasons. Rates of N cycling are low relative to other alpine communities (Fisk et al. 1998), and thus dry meadows may be more sensitive to ecological impacts of N deposition. Previous work indicated plant production in dry meadows is N limited, but that community-level growth response to N fertilization is determined by changes in species composition rather than increased biomass of the dominant species (Bowman et al. 1993, 1995). Plant species density in dry meadows is relatively high (10–30 species/m\(^2\)), increasing the probability of a response in diversity with increasing N availability. Thus dry meadows may be the most sensitive community to biotic changes resulting from N deposition within the alpine landscape.

**Experimental procedures**

The experimental treatments consisted of three levels of N fertilization (20, 40, and 60 kg N ha\(^{-1}\) yr\(^{-1}\), roughly equaling 3\(\times\), 6\(\times\), and 9\(\times\) greater N inputs than current levels of N deposition on Niwot Ridge, along with a control (ambient total wet + dry deposition, 6 kg N ha\(^{-1}\) yr\(^{-1}\) [Sievering 2001]) treatment. The range in N inputs was used to provide dose-specific estimates of the responses of vegetation and soils. The N was applied as NH\(_4\)NO\(_3\) in aqueous solution (5 L each plot), three times during the growing season using backpack sprayers, with...
half added in the first application in late May to early June, as soon as the plots became snow free, and the remaining half split between the next two applications, added in late June and mid-July. This application procedure was followed since approximately half of the N deposition occurs in winter and is associated with N leaching through snowpack (Bowman 1992). The addition of water to the plots represented a 10-mm increase in precipitation, or approximately a 6% increase in summer precipitation, well within the range of interannual variability. The treatments were applied to five replicate 1 × 1.5 m plots for each treatment for a total of 20 plots, arranged in five replicate blocks. A 1 × 0.5 m portion of the plot was reserved for destructive soil sampling, while the remainder was used for monitoring vegetation change. The plots were established in 1997, and the treatments were applied each year.

The composition of vascular plant species within the plots was measured each year (except 2001) using a point-intercept method with a 10 × 10 grid of 100 points in each plot. Species nomenclature follows Weber (1976). Species that occurred within a plot but not recorded at one of the points were given a projected cover value of 0.5. Because the leaf area index was sometimes >1, >100 points were recorded in some plots. Species richness (number of species per square meter) and H’, the Shannon-Wiener index of diversity, which incorporates both richness and evenness of species, were recorded for each plot in each year. In addition, the compositional similarity of vegetation for all plots in all years was estimated using a detrended correspondence analysis (DCA), using PC-ORD (MJM Software, Gleneden Beach, Oregon, USA).

To examine the response of aboveground production to the N treatments, as well as to estimate plant uptake of N, aboveground biomass was clipped in 3 × 0.04 m² subplots within each of the larger plots. Biomass was clipped at peak season growth, which occurred between late July to early August, in 1998, 2002, and 2004. The biomass was sorted according to the major growth forms found in the plots, including Kobresia, all other graminoids, and all forbs. In 2002 and 2004 the graminoid component was split into Carex species and grasses. After collection, the biomass was oven-dried to constant mass at 70°C for 48 h. Dry mass was recorded for each of the growth forms to the nearest 0.01 g. The aboveground biomass collected in 1998 was analyzed for tissue N concentration using a Kjeldahl analysis after acid digestion, followed by colorimetric analysis using a Lachat QuikChem 8000 Spectrophotometric Flow Injection Analyzer (Lachat, Milwaukee, Wisconsin, USA). Biomass collected in 2002 and 2004 was analyzed for N concentration using a CHN autoanalyzer coupled with an isotope-ratio mass spectrometer (at the University of California, Davis Stable Isotope Facility; Europa Integra, Cheshire, UK) used for 15N analysis of the biomass. Aboveground tissue N content was calculated as the product of N concentrations and aboveground biomass for each biomass component. Data for aboveground biomass and N accumulation for the 1998 harvest were presented in an earlier publication (Bowman 2000).

In 2002, a 15N tracer was applied to the plots to evaluate the important ecosystem sinks for N deposition in dry meadows (K. Holland, A. R. Townsend, and W. D. Bowman, unpublished manuscript). Here we report only on the uptake of 15N by the plant growth forms, to evaluate the importance of compositional changes for plant N uptake. The 15N tracer was added as 98.5% enriched 15NH4+15NO3 at a low level (total 0.027 g N·m⁻²·yr⁻¹) to minimize any additional fertilization effect. The 15N tracer was added in solution to the canopy in three applications during the growing season (mid-June, early July, and early August). Dried, ground, and homogenized subsamples of the biomass harvests from the 2002 and 2004 harvests were analyzed for 15N abundance at the University of California, Davis Stable Isotope Facility. Tissue 15N enrichment and percentage of recovery were calculated using equations found in Hauck and Brenner (1976).

Soil inorganic N concentrations in the plant rooting zone (2–10 cm depth) were measured in 1999, 2001, and 2003 using microlysimeters, 2.5 mm diameter × 10 cm length tubes constructed from a hydrophilic porous polymer (Rhizon soil moisture samplers, Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands). Two microlysimeters per plot were deployed, for a sample size of 10 per treatment. Soil water samples were collected early in the growing season, prior to the first application of fertilizer. Sample collection was typically limited to the first third of the growing season, as drying soils prevented collection in the later part of the summer. Samples were collected by placing vacuumers on the microlysimeters for 12–24 h. Vacuumers were collected from the field and frozen until they were analyzed for inorganic N concentrations, usually within 1–2 mo. Soil water was analyzed for NH4+15N and NO3⁻15N in the Kiowa Chemistry Laboratory at the Mountain Research Station, using a Lachat QuikChem 8000 Spectrophotometric Flow Injection Analyzer and a Dionex DX 500 System IonPac AS11 Ion Chromatograph (Sunnyvale, California, USA), respectively.

Inorganic N leaching below the rooting zone was assessed using ion exchange resin bags (Binkley and Vitousek 1989). Mixed-bed ion exchange resins (J.T. Baker, IONAC NM-60 H⁺/OH⁻; Phillipsburg, New Jersey, USA) were placed in fine mesh nylon bags, surrounded by a plastic cylinder (4.9 cm², ~25 mm height) to maintain a rigid structure in the soil. Two bags were placed in the soil within each treatment plot during the first week of June in 2001 (fifth year of the experiment), prior to fertilizer application. The bags were inserted into the soil at a depth of 15 cm, below the depth of maximum soil root density (10 cm) in alpine dry meadows (Webber and May 1977), by removing a plug of intact soil and sod and inserting the resin bags into the soil at the edge of the hole such that the column
of soil above the bag remained undisturbed. The resin bags were retrieved at the end of July, after 52 d. A subset of resin bags not deployed in the field were used as blanks. Within 4 h of collection, the resin bags were brought back to the soils laboratory at the Mountain Research Station, composited into a single sample for each plot, and extracted with 2 mol/L KCl. The extractant was analyzed for NH₄⁺ and NO₃⁻ as described above.

Changes in soil N cycling rates due to the N fertilization treatments were measured using buried bag incubations during the seventh year of the experiment, in 2003. Soil cores (3.5 cm diameter × 10 cm depth) were collected early in the growing season (9 June), prior to the first application of the fertilization treatment. The cores were gently vertically split and placed into two plastic bags, one placed back into the soil and covered with loose soil and plant litter and the other brought back to the soils laboratory at the Mountain Research Station. Two cores were collected in each plot and composited in the laboratory after collection. The field-collected soil was sieved to 2 mm to remove rocks and coarse plant tissues and split into two 10-g subsamples, one extracted using 2 mol/L KCl, the other for determination of the fresh/dry mass ratio after oven-drying at 105°C for 48 h. The soil extractant was analyzed for NH₄⁺-N and NO₃⁻-N in the Kiowa Laboratory as described above. The incubated samples were retrieved after 22 d and processed and analyzed for NH₄⁺-N and NO₃⁻-N as above. Net N mineralization and nitrification were calculated as the difference in total inorganic N and NO₃⁻-N concentrations between the incubated and initial samples, respectively.

The vegetation data (species density, H', abundance of individual species, and DCA scores) were analyzed using repeated-measures analysis of variance (Wilks' lambda). The effect of the N treatments on aboveground biomass, foliar N concentrations and contents, ¹⁵N recovery and tissue enrichments, soil inorganic N concentrations, and rates of net N mineralization and nitrification were analyzed using a general linear model ANOVA, with block included as a categorical variable when significant. Transformations (log, arcsine) were used in a few cases when the data distributions did not meet the assumptions of the tests.

**RESULTS**

**Vegetation responses: composition and biomass**

Species richness in the plots ranged from 12 to 29 species/m² (20 ± 0.3 species/m² [means ± se]). There were significant differences among years, but no treatment or treatment × year effects on species richness (Table 1). Diversity, incorporating both richness and species abundance using the Shannon-Wiener index \(H'\), increased significantly with the N addition treatments (Fig. 1A, Table 1). By the fourth year of the experiment diversity was higher at all levels of the N treatments relative to the control. The change in diversity was due primarily to an increase in the cover of Carex rupestris (Fig. 1B, Table 1). Other species also contributed to the increase in diversity through increased abundance, although only Trisetum spicatum occurred in enough plots for this to be statistically significant (Table 1). There were no significant negative trends in species composition for any species over the eight years of the experiment.

A detrended correspondence analysis (DCA) was performed to evaluate the change in compositional similarity of plots through time. The first axis of the analysis had an eigenvalue of 0.095 and explained 26% of the variance based on determination of the relative Euclidean distance in the ordination space. The influence of nitrogen addition on changes in DCA1 score for each individual plot was examined through time using a repeated-measures ANOVA. The DCA1 scores in the N treatment plots decreased with time (Fig. 1C, Table 1), indicating that species composition changed through time as a result of increased N availability.

To estimate the critical load of N that would cause a significant change in vegetation composition, we plotted the rate of change in the cover of Carex rupestris and the DCA1 scores for each plot in each N treatment. This approach assumed any change in community composition at ambient conditions was nil, or if significant, due to N deposition (Fig. 2). To objectively estimate the level of N input that would result in a significant change in
vegetation in the alpine dry meadow (x-intercept) we fit these relationships with a sigmoidal dose response curve for the change in DCA and a hyperbola for rate of change in Carex cover, which assumed that the rate of change in vegetation composition would saturate at higher levels of N addition. Using the current deposition rate of 6 kg N ha$^{-1}$yr$^{-1}$ for the ambient control, we estimated that inputs between 4 (based on the change in Carex cover) and 10 (based on changes in the DCA1 score) kg N ha$^{-1}$yr$^{-1}$ would elicit a significant change in dry-meadow species composition. The estimate of the N level that would cause changes in $H'$ gave an N critical load lower than 0 kg N ha$^{-1}$yr$^{-1}$, possibly indicating multiple factors contributing to an ongoing increase in diversity in our control plots.

Aboveground biomass in the dry meadow increased significantly in response to the N treatments in 2002, but not in 1998 or 2004 (Fig. 3A, Table 2). The increase in total biomass in 2002 was the result of significant increases in forb and Carex biomass. Although the total biomass did not change in 1998 and 2004, individual components of the biomass did change significantly with increasing N. There were significant increases in the graminoid (including Carex) biomass in 1998 and the Carex biomass in 2004. In 2004, there was a significant decrease in Kobresia biomass with increasing N input. The interannual variation in biomass production was pronounced, with lower biomass in 2002 and 2004 potentially reflecting a significant drought in 2002.

Nitrogen addition increased tissue N concentrations in all growth forms in all years, except for Carex and grass in 2004 (Tables 2 and 3). Total pools of plant N increased with N additions in all years (Fig. 3B, Table 2). The increase was due to larger N pools in forbs and graminoids (including Carex) in 1998 and in forbs and

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**FIG. 1.** Responses of vegetation composition in an alpine dry meadow to three nitrogen treatments (additions of 20, 40, and 60 kg N ha$^{-1}$yr$^{-1}$) (means ± se) and an ambient control treatment over an 8-yr period. (A) $H'$, the Shannon-Wiener index, incorporating both species richness and evenness, for vascular plant species; (B) cover of Carex rupestris, the species that responded most strongly; and (C) scores for the first axis of a detrended correspondence analysis (DCA1), indicating the similarity of species composition within the plots. Significant changes in all of these indicators of composition occurred as a result of the N treatment. The research was carried out in an alpine dry meadow at 3500 m on Niwot Ridge, Colorado, USA.

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**FIG. 2.** Estimation of the N critical load for changes in vegetation composition in an alpine dry meadow, using changes in the rates of (A) increase in Carex rupestris cover and (B) decrease in scores for the first axis of a detrended correspondence analysis (DCA1) as a function of increasing N inputs (means ± se). Data were fit with a hyperbolic function for (A) and a sigmoidal dose-response curve for (B) ($R^2 = 0.99$). Projections of these functions to the x-intercept, along with ambient N deposition estimate of 6 kg N ha$^{-1}$yr$^{-1}$, gave a range in N critical loads of between 4 and 10 kg N ha$^{-1}$yr$^{-1}$.
Carex in 2002 and 2004. Kobresia, the dominant in the community, did not show significant increases in N pools in any year.

The uptake of $^{15}$N (tissue enrichment) varied among the growth forms and among the treatments (Fig. 4A). Tissue enrichment was highest in Carex biomass, intermediate in grass and forb biomass, and lowest in Kobresia. Similar patterns were found for the 2004 harvest, although the amount of tissue enrichment was lower (data not shown). Tissue $^{15}$N enrichments decreased with increasing N addition in Carex biomass. The recovery of the $^{15}$N label, incorporating both tissue enrichment and total biomass, did not change with the N treatment and was lowest in the grass component, intermediate in forb biomass, and highest in the Kobresia and Carex components (Fig. 4B).

Soil responses: inorganic N and N cycling

Concentrations of NH$_4^+$ in soil solution did not vary with the N addition treatments in any of the years (data not shown, values <1 mg N/L). NO$_3^-$ concentrations did not increase with N additions in 1999 ($F_{3,43} = 1.207$, $P = 0.319$), but were significantly higher in 2001 ($F_{3,19} = 3.676$, $P = 0.030$) and 2003 ($F_{3,11} = 3.549$, $P = 0.051$; Fig. 5A). A significant increase in NO$_3^-$ concentrations occurred with addition of N at or greater than 40 kg·ha$^{-1}$·yr$^{-1}$. Inorganic N captured in resin bags below the rooting zone followed a similar pattern as the inorganic N in the rooting zone (Fig. 5B). No significant change in NH$_4^+$ occurred in the N addition plots ($F_{3,34} = 1.343$, $P = 0.277$), while NO$_3^-$ increased ($F_{3,34} = 3.433$, $P = 0.028$). Likewise, a significant increase in NO$_3^-$ occurred only at 60 kg·N·ha$^{-1}$·yr$^{-1}$ treatment.

Net N mineralization rate did not vary among the treatments ($F_{3,16} = 1.542$, $P = 0.247$), but net nitrification rate increased with increasing N addition ($F_{3,16} = 6.377$, $P = 0.024$), although only at 40 kg·N·ha$^{-1}$·yr$^{-1}$ (Fig. 6).

**DISCUSSION**

Vegetation composition was altered at lower N inputs than the levels at which measurable changes in soil inorganic N and nitrification occurred in the alpine dry meadow. Changes in diversity and community composition occurred at all levels of N input, while changes in the soil indicators of N status that we measured occurred at levels equal to or higher than 40 kg·N·ha$^{-1}$·yr$^{-1}$. Part of the difference in the apparent responses to N inputs between the vegetation and soil may be in our ability to detect such changes. Soil properties are dynamic in space and time, making detection of trends more difficult, while temporal changes in vegetation composition tend to be more sustained, particularly for perennial plants of the alpine. Thus, species composition appears to be a more reliable and possibly more sensitive indicator than changes in soil inorganic N or N cycling for ecological effects of N deposition in the vegetated component of the alpine landscape. This is the first study of which we know explicitly comparing the relative responses of plant...
species composition and soil N properties as indicators of N critical loads using a long-term field experiment. Given that changes in soil N status are potential precursors for more harmful ecosystem effects such as loss of base cations and soil and surface water acidification (Aber et al. 1998), the use of vegetation composition as an indicator of N critical loads may be preferred in sensitive terrestrial ecosystems found in wilderness areas and national parks, which have been designated by the U.S. Congress as Class I areas (Burns 2003).

We estimated the N critical load for vegetation change in our alpine dry meadow at 4 kg N-ha\(^{-1}\)-yr\(^{-1}\), using the response rate of individual species changes to increases in N input, and 10 kg N-ha\(^{-1}\)-yr\(^{-1}\), using the response rate of the community to N inputs (Fig. 2). These estimates are within the ranges of other estimates of N critical loads for N saturation of surface waters derived from modeling and whole catchment studies for Niwot Ridge (4 kg N-ha\(^{-1}\)-yr\(^{-1}\) [wet deposition only]; Williams and Tonnessen 2000) and nearby Loch Vale (3–4 kg N-ha\(^{-1}\)-yr\(^{-1}\); Baron et al. 1994), as well as the N critical loads for vegetation change proposed for alpine sites in Europe on acidic soils (3–15 kg N-ha\(^{-1}\)-yr\(^{-1}\); Reynolds et al. 1998, Bobbink et al. 2002). Our estimates of N critical loads for vegetation change are consistent with estimates derived from modeling and whole catchment studies for Niwot Ridge (4 kg N-ha\(^{-1}\)-yr\(^{-1}\) [wet deposition only]; Williams and Tonnessen 2000) and nearby Loch Vale (3–4 kg N-ha\(^{-1}\)-yr\(^{-1}\); Baron et al. 1994), as well as the N critical loads for vegetation change proposed for alpine sites in Europe on acidic soils (3–15 kg N-ha\(^{-1}\)-yr\(^{-1}\); Reynolds et al. 1998, Bobbink et al. 2002). Our estimates of N critical loads for vegetation change are consistent with estimates derived from modeling and whole catchment studies for Niwot Ridge (4 kg N-ha\(^{-1}\)-yr\(^{-1}\) [wet deposition only]; Williams and Tonnessen 2000) and nearby Loch Vale (3–4 kg N-ha\(^{-1}\)-yr\(^{-1}\); Baron et al. 1994), as well as the N critical loads for vegetation change proposed for alpine sites in Europe on acidic soils (3–15 kg N-ha\(^{-1}\)-yr\(^{-1}\); Reynolds et al. 1998, Bobbink et al. 2002). Our estimates of N critical

### Table 2. ANOVA for the effect of nitrogen addition on biomass accumulation, tissue N concentration, and N content for plant growth forms.

<table>
<thead>
<tr>
<th>Growth form, by year</th>
<th>Biomass</th>
<th>N concentration</th>
<th>N content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(F_{1,14})</td>
<td>(P)</td>
<td>(F_{1,14})</td>
</tr>
<tr>
<td><strong>1998</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Kobresia</em></td>
<td>0.639</td>
<td>0.427</td>
<td>45.75</td>
</tr>
<tr>
<td>Forb</td>
<td>1.04</td>
<td>0.312</td>
<td>55.59</td>
</tr>
<tr>
<td>Graminoid</td>
<td>13.97</td>
<td>0.004</td>
<td>67.01</td>
</tr>
<tr>
<td>Total</td>
<td>2.23</td>
<td>0.142</td>
<td>16.90</td>
</tr>
<tr>
<td><strong>2002</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Kobresia</em></td>
<td>0.533</td>
<td>0.668</td>
<td>11.03</td>
</tr>
<tr>
<td>Forb</td>
<td>4.90</td>
<td>0.011</td>
<td>16.01</td>
</tr>
<tr>
<td>Carex</td>
<td>18.75</td>
<td>0.001</td>
<td>4.71</td>
</tr>
<tr>
<td>Grass</td>
<td>1.715</td>
<td>0.211</td>
<td>10.17</td>
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<tr>
<td>Total</td>
<td>14.52</td>
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</tr>
<tr>
<td><strong>2004</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Kobresia</em></td>
<td>6.558</td>
<td>0.023</td>
<td>78.87</td>
</tr>
<tr>
<td>Forb</td>
<td>2.855</td>
<td>0.113</td>
<td>21.20</td>
</tr>
<tr>
<td>Carex</td>
<td>11.607</td>
<td>0.004</td>
<td>0.71</td>
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<tr>
<td>Grass</td>
<td>2.869</td>
<td>0.112</td>
<td>1.682</td>
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<tr>
<td>Total</td>
<td>0.270</td>
<td>0.011</td>
<td>11.759</td>
</tr>
</tbody>
</table>

**Notes:** Treatments consisted of three levels of nitrogen addition (20, 40, and 60 kg N-ha\(^{-1}\)-yr\(^{-1}\)) and an ambient control treatment. Biomass was harvested at peak season growth in 1998, 2002, and 2004 (n = 5 plots per treatment, except for grass, which varied from 1 to 3).

### Table 3. Foliar N concentrations (in percentages) for aboveground biomass of plant growth forms in plots subjected to nitrogen addition vs. control.

<table>
<thead>
<tr>
<th>Growth form, by year</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1998</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Kobresia</em></td>
<td>1.82(\pm) 0.07</td>
<td>2.00(\pm) 0.05</td>
<td>2.23(\pm) 0.06</td>
<td>2.21(\pm) 0.06</td>
</tr>
<tr>
<td>Forb</td>
<td>1.59(\pm) 0.05</td>
<td>1.81(\pm) 0.05</td>
<td>2.16(\pm) 0.08</td>
<td>2.23(\pm) 0.08</td>
</tr>
<tr>
<td>Graminoid</td>
<td>1.37(\pm) 0.07</td>
<td>1.70(\pm) 0.07</td>
<td>1.82(\pm) 0.13</td>
<td>2.02(\pm) 0.05</td>
</tr>
<tr>
<td><strong>2002</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Kobresia</em></td>
<td>1.71(\pm) 0.09</td>
<td>2.15(\pm) 0.03</td>
<td>2.10(\pm) 0.17</td>
<td>2.36(\pm) 0.13</td>
</tr>
<tr>
<td>Forb</td>
<td>1.76(\pm) 0.09</td>
<td>1.97(\pm) 0.11</td>
<td>2.43(\pm) 0.22</td>
<td>2.48(\pm) 0.16</td>
</tr>
<tr>
<td>Carex</td>
<td>1.71(\pm) 0.12</td>
<td>1.77(\pm) 0.07</td>
<td>1.73(\pm) 0.07</td>
<td>2.00(\pm) 0.10</td>
</tr>
<tr>
<td>Grass</td>
<td>1.41</td>
<td>1.85(\pm) 0.05</td>
<td>1.89(\pm) 0.21</td>
<td>2.32(\pm) 0.17</td>
</tr>
<tr>
<td><strong>2004</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Kobresia</em></td>
<td>1.76(\pm) 0.07</td>
<td>2.07(\pm) 0.06</td>
<td>2.50(\pm) 0.08</td>
<td>2.57(\pm) 0.05</td>
</tr>
<tr>
<td>Forb</td>
<td>1.88(\pm) 0.15</td>
<td>2.10(\pm) 0.07</td>
<td>2.32(\pm) 0.08</td>
<td>2.93(\pm) 0.06</td>
</tr>
<tr>
<td>Carex</td>
<td>1.92(\pm) 0.16</td>
<td>1.95(\pm) 0.03</td>
<td>2.08(\pm) 0.05</td>
<td>2.03(\pm) 0.03</td>
</tr>
<tr>
<td>Grass</td>
<td>1.77(\pm) 0.05</td>
<td>1.94</td>
<td>2.32(\pm) 0.19</td>
<td>2.33(\pm) 0.19</td>
</tr>
</tbody>
</table>

**Notes:** Treatments consisted of three levels of nitrogen addition (20, 40, and 60 kg N-ha\(^{-1}\)-yr\(^{-1}\)) and an ambient control treatment. Biomass was harvested at peak season growth in 1998, 2002, and 2004. Values are means ± se (n = 5 plots per treatment, except for grass, which varied from 1 to 3).
loads are empirically based on a relatively long-term experimental addition of N. However, N deposition has been increasing for many decades, and our critical loads may be higher than what would elicit an ecological change over many decades. It is likely that almost any level of N increase will have a significant impact on sensitive alpine biota when sustained over five decades.

These N critical load estimates suggest that the current rate of N deposition (4–6 kg ha⁻¹ yr⁻¹) may be impacting ecological processes in the Colorado Front Range. Several lines of evidence support N deposition in the Front Range as the cause of significant environmental changes (Baron et al. 2000). Changes in the diatom composition in alpine lakes in Rocky Mountain National Park have occurred in conjunction with the temporal pattern in increases in N deposition in the Front Range (Wolfe et al. 2001) and in a manner consistent with experimental manipulation of lake N status (McKnight et al. 1990). C:N ratios in needles and soil of old-growth forests are significantly lower and rates of net N mineralization are higher in stands on the eastern side relative to the western side of the continental divide, consistent with higher rates of N deposition at these sites (Baron et al. 2000, Rueth et al. 2003). Significant increases in nitrophilous plant species were noted over the past two decades in long-term monitoring plots on Niwot Ridge (Korb and Ranker 2001). Finally, episodic loss of acid-neutralizing capacity has been recorded in talus-dominated catchments in the uppermost Green Lakes Valley below Niwot Ridge (Caine 1995, Williams and Tonnesen 2000). Taken together, these studies, along with our empirically derived N critical load, indicate that ecological changes are occurring in the alpine and subalpine of the Colorado Front Range as a direct result of anthropogenic N deposition. These studies also indicate that our estimate of N critical loads for vegetation and soils are relatively conservative, since multi-decadal N deposition inputs at levels lower than our critical loads appear to have elicited significant ecological changes.

**FIG. 4.** (A) Atom percent ¹⁵N enrichment and (B) the percentage of ¹⁵N recovery in component growth forms receiving trace amounts of enriched ¹⁵N, growing in plots in an alpine dry meadow receiving three nitrogen treatments (additions of 20, 40, and 60 kg N ha⁻¹ yr⁻¹) and an ambient control treatment (means and se). Significant differences occurred in atom percent ¹⁵N enrichment among growth forms (Carex > grass, forb > Kobresia), and Carex enrichment decreased with increasing N input. The recovery of labeled ¹⁵N varied by growth form (Kobresia, Carex > forb > grass) and did not vary with N treatment.

![Diagram](image-url)
The change in vegetation composition was determined in large part by an increase in the cover of a single species of sedge, *Carex rupestris*, as well as several grass species, most notably *Trisetum spicatum*. These species exhibited greater uptake of $^{15}$N, and in a previous study they had greater growth responses to changes in N availability than the dominant sedge *Kobresia myosuroides* (Bowman and Bilbrough 2001). Increases in the cover of these responsive species, without a concomitant decrease in other species, resulted in higher evenness (and thus diversity) with increasing N inputs. The increase in diversity contrasts with species compositional responses in other herbaceous communities, which are often marked by the replacement of several species by a lower number of nitrophilous species, typically grasses, leading to a decrease in diversity (e.g., Van Dam et al. 1986, Bobbink et al. 1998, Gough et al. 2000, Zavaleta et al. 2003). Increases in diversity have occurred in previous fertilization studies in alpine dry meadows (Theodose and Bowman 1997), although these were shorter term studies with higher doses of N added, and a significant decrease in cover of the dominant species was noted. Nutrient supply to plants in the alpine is low (Fisk et al. 1998), and thus increases in N supply may allow more species to coexist over the short term (decadal time periods). Eventually diversity may decline as species dominance in the community shifts to a few nitrophilous species, consistent with a unimodal distribution of diversity across a resource availability gradient (Rosenzweig and Abramsky 1993, Bowman and Damm 2002).

Increases in plant N uptake with increasing N inputs, as evidenced both by N pools and $^{15}$N recovery, were determined primarily by increased tissue N concentrations and changes in species composition, rather than increases in overall community biomass. The increase in aboveground biomass was significant in only one of the three years measured, and the amount of increase was modest relative to the interannual variability. Changes in the component growth forms of community biomass, particularly increases in *Carex*, also contributed to the greater aboveground N pools, although this increase was offset by a significant decrease in *Kobresia* biomass in 2004. In general the increase in plant N pools was large relative to other ecosystems, particularly forest systems (Nadelhoffer et al. 1995, Magill et al. 2000, Zak et al. 2004, Templer et al. 2005), where plants are typically a smaller sink for external N inputs relative to bulk soils. Increases in the aboveground pools of N accounted for 29% (1998) to 38% (2004) of the N applied in the plots in the 2 g N m$^{-2}$ yr$^{-1}$ treatment, with generally lower proportions in the higher N treatments. Belowground plant tissues also potentially sequestered some of the added N, primarily through increases in tissue N concentration, since production increases in belowground biomass is lower or nil under N fertilization (Bowman et al. 1993). Recovery of $^{15}$N in roots was higher than aboveground tissues, and root N concentrations increased with the N treatment, indicating belowground tissues are an important pool for N inputs (K. Holland, A. R. Townsend, and W. D. Bowman, unpublished manuscript). Plants therefore appear to be a relatively important component of the initial N sequestration of atmospheric N deposition in the alpine.

Changes in plant species composition, coupled with changes in litter C:N ratios associated with increasing N deposition (K. Holland, A. R. Townsend, and W. D. Bowman, unpublished manuscript) are likely to limit the capacity of plants and associated soil organic matter to remain significant long-term sinks for N. Increases in species with higher tissue turnover (particularly fine roots), along with lower C:N ratios of plant litter, may stimulate rates of N cycling and N losses through leaching (Bowman and Steltzer 1998). We measured increases in net nitrification rates, but no changes in net N mineralization rates, and thus these hypothesized changes are modest at this point in time. However, soils collected from the N-fertilized plots show lower retention of $^{15}$N than the control plots, indicating more rapid N turnover, probably within the more labile, lighter fraction of soil organic matter (K. Holland, A. R. Townsend, and W. D. Bowman, unpublished manuscript). These changes in the dynamics of the soil organic pool are consistent with changes in light and heavy soil organic matter fractions measured in soils in alpine dry meadows receiving higher amounts of N fertilization for over a decade (Neff et al. 2002).

In addition to plant species and microbial constraints on long-term plant sequestration of N deposition, changes in plant–herbivore interactions may also limit the capacity of plants as a long-term N pool. Higher tissue N concentrations, coupled with potential changes in plant secondary chemistry (lower production of phenolics [Throop and Lerdau 2004]), may enhance generalist herbivore population growth (Dearing 2000), increase rates of herbivory, and lower storage of N in soil organic matter pools.
Conclusions

There is certainty that reactive N concentrations in air and deposition in the western United States will increase with increasing emissions from both point and nonpoint sources, related to increases in population, energy development, and agricultural activities (Baron et al. 2000, Fenn et al. 2003b). Environmental effects of N deposition have been noted throughout the West, and it has been suggested that much of this region is more susceptible to impacts from N deposition than more mesic and productive systems, such as forested ecosystems in the eastern United States (Fenn et al. 2003a). We have estimated N critical loads for the response of individual species and communities (4 and 10 kg N·ha⁻¹·yr⁻¹, respectively) and soil (>20 kg N·ha⁻¹·yr⁻¹) for alpine dry meadows in the Front Range of the Colorado Rockies that substantiate the concern for changes in biological resources in Class I areas (wilderness areas and national parks). Our results also suggest that the changes in alpine plant species composition recorded on Niwot Ridge (Korb and Ranker 2001; K. N. Suding and W. D. Bowman, unpublished data) are in response to N deposition, similar to changes in lake phytoplankton recorded by Wolfe et al. (2001). Therefore, in answer to the question, “are we there yet?” in terms of N critical loads for vegetation, the answer appears to be “yes.” Finally, this research indicates that vegetation changes will precede detectable changes in soil inorganic N concentrations (and in fact, buffer them to a degree) and N cycling (and eventually may be the cause for such changes), and thus monitoring vegetation, or other sensitive biota, may be the preferred metric for determining ecological responses to N deposition.

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Literature Cited


