

Limited ecosystem recovery from simulated chronic nitrogen deposition

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Abstract. The realization that anthropogenic nitrogen (N) deposition is causing significant environmental change in many ecosystems has led to lower emissions of reactive N and deposition rates in many regions. However, the impacts of N deposition on terrestrial ecosystems can be long lasting, with significant inertia in the return of the biota and biogeochemical processes to baseline levels. To better understand patterns of recovery and the factors that may contribute to slow or no responses following declines in N deposition, we followed plant species composition, microbial abundance, N cycling rates, soil pH, and pools of NO_3^- and extractable cations in an impacted alpine ecosystem following cessation of 12-yr experiment increasing N deposition rates by 0, 20, 40, and 60 $\text{kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$. Simulated N deposition had resulted in a tripling in the cover of the nitrophilic species *Carex rupestris*, while the dominant sedge *Kobresia myosuroides* had decreased by more than half at the highest N input level. In addition, nitrification rates were elevated, soil extractable magnesium (Mg^{2+}) and pH decreased, and aluminum (Al^{3+}) and manganese (Mn^{2+}) were elevated at the highest N treatment inputs. Over the nine years following cessation of N additions to the impacted plots, only the cover of the nitrophilic *C. rupestris* showed any recovery to prior levels. Abundances of both bacteria and fungi were lower with N addition in both treatment and recovery plots. Rates of nitrification and pools of NO_3^- remained elevated in the recovery plots, likely contributing to the lack of biotic response to the cessation of N inputs. In addition, nutrient base cations (Ca^{2+} and Mg^{2+}) and soil pH remained depressed, and the toxic metal cations (Al^{3+} and Mn^{2+}) remained elevated in recovery plots, also potentially influencing biotic recovery. These results emphasize the importance of considering long-term environmental impacts of N deposition associated with legacy effects, such as elevated N cycling and losses of base cations, in determining environmental standards such as the metrics used for critical loads.

Key words: alpine; base cation depletion; ecosystem recovery; microbial abundance; nitrate pools; nitrification; nitrogen deposition; plant species composition; soil acidification; toxic cations.

INTRODUCTION

Anthropogenic creation of reactive N and subsequent release into the biosphere, both intentional and nonintentional, has led to substantial environmental impacts in addition to increases in agricultural yields (Vitousek et al. 1997, Galloway et al. 2008). Atmospheric deposition of N into terrestrial ecosystems has resulted in enhancement of forest growth (Thomas et al. 2010), a loss of plant diversity (Stevens et al. 2004, Bobbink et al. 2010, Simkin et al. 2016), depletion of nutrient base cations, and subsequent acidification and enhancement of toxic soluble aluminum (Al) and manganese (Mn) in soils and surface waters (van Breemen et al. 1983, Driscoll et al. 2001). The expected temporal trend of ecosystem responses to N deposition inputs, based primarily on experimental additions of N, begins with increases in net primary production (NPP). Subsequently, plant diversity and microbial biomass often decrease in association with ecosystem eutrophication, followed by enhanced rates of N cycling linked with decreased carbon:N of ecosystem pools. As inputs of N and cycling rates increase, greater NO_3^- leaching occurs, leading to loss of base cations, greater

soluble Al in soil solution, decreased pH, contributing to decreases in NPP (Aber et al. 1998, Lovett and Goodale 2011, Bowman et al. 2015, Clark et al. 2017).

Although N emission rates have decreased and rates of deposition have leveled off or declined in some developed regions such as Europe and the eastern United States (Du et al. 2014, Waldner et al. 2014), emissions are expected to increase in developing countries (Galloway et al. 2008, Liu et al. 2013). Even if inputs from N deposition decline, the impacts may be long-lasting (Groffman et al. 2006). The further along an ecosystem is in the trajectory of impacts described above, including eutrophication and acidification, the less likely that ecosystem is to show recovery once N deposition rates are lowered. Multiple results from both observational and experimental studies indicate that ecosystem recovery, or the return of biotic composition and ecosystem processes to pre-impacted conditions with decreases in N deposition, occurs slowly or not at all, even after a decade or more of lowered N inputs (Bowman et al. 1998, Strengbom et al. 2001, Clark et al. 2009, and others reviewed in Stevens 2016). The potential for the recovery from the impacts of N deposition may be slowed due to the enrichment of ecosystem pools of N (Vinton and Burke 1995), and changes in plant and microbial communities that may enhance rates of N cycling and continue to elevate soil N supply (Bowman and Steltzer 1998, Freedman et al.

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2016, Choma et al. 2017) and resist the re-invasion of the community by the original species by occupying the space (Harris et al. 2011). Furthermore, geochemical changes in the soil such as greater weathering of Al at low soil pH and leaching of soil cations may take decades or more to reverse (Nohrstedt 1998, Wright et al. 2001), although some studies have reported significant recoveries of soil base cations within a decade of recovery (Högberg et al. 2006). While there is abundant evidence for limited reversibility of the effects of N deposition on ecosystem processes, few studies have examined the simultaneous recovery of plant community composition, microbial abundance, and ecosystem processes to compare their relative responses to lowered N deposition. Such information is important to better understand the long-term impacts of N deposition and for selecting appropriate critical loads for protecting natural resources.

To address the reversibility of impacts associated with N deposition in a sensitive alpine ecosystem, we split treatment plots after 12 yr of an N addition experiment and ceased fertilizing one-half of each plot, but continued adding N to the other half at the same rate. We followed the changes in biotic composition and ecosystem processes for nine more years in both treated and untreated subplots. During the first decade of the experiment (Bowman et al. 2006, Lieb et al. 2011) we found that increases in NPP were minimal and transient, but that plant foliar pools of N increased with simulated N deposition due to increases in tissue N concentrations. One nitrophilic species, *Carex rupestris*, increased in cover significantly at rates around 4 kg N/ha (lower than the current ambient deposition rates). The dominant sedge, *Kobresia myosuroides*, decreased in abundance and overall community composition was altered at rates of N deposition above 10 kg N/ha. Nitrification rates and leaching of NO_3^- increased beyond additions of 20 kg N/ha. At the highest inputs of N (40–60 kg N/ha) loss of nutrient base cations and acid buffering capacity occurred with concomitant decreases in soil pH and increases in toxic soluble Al and Mn.

We hypothesized that recovery following cessation of N additions would be most apparent for plant species composition, particularly the abundance of the nitrophilic indicator species *C. rupestris*, which we expected to decrease. Higher individual plant and population growth rates of nitrophilic species would make them more likely to respond to both increases and decreases in soil N supply. We also expected that the abundances of bacteria and fungi would both decrease with N addition, but more so for bacteria, and that the abundances of both groups would increase following cessation of the treatment. Due to the toxic effects of higher soluble Al and Mn, we expected the treatments that have received the highest N inputs would exhibit little or no recovery of both plant species and microbial abundance. We hypothesized that N cycling rates and labile soil N pools would decrease in recovery plots relative to treatment plots. Finally, we hypothesized that the recovery of soil base cations and pH would be minimal or nonexistent, due to these factors being dependent on mineral weathering rates and the potential continued impacts from elevated N cycling rates in soils.

METHODS

Experimental design

A long-term N deposition simulation experiment was started in an alpine dry meadow community at 3,500 m on Niwot Ridge in 1997 (details in Bowman et al. 2006). Climate at the research site is cold (average annual temperature of -4°C) with annual precipitation of 990 mm falling mainly as snow in the winter. The growing season lasts for 8–10 weeks. Soils are Cryumbrepts derived from granitic parent material, with contributions of dust derived from arid regions to the west (Muhs and Benedict 2006, Neff et al. 2008). Ambient total measured wet + dry N deposition at the site is 6 kg N/ha.

To estimate thresholds of N deposition input that would elicit specific responses in plant community composition, N cycling and pools, and soil buffering capacity, in our original experiment, we applied three levels of N (20, 40, and 60 kg N/ha, applied as NH_4NO_3 in solution, three times during the growing season, in early to mid June, early July, and late July) in addition to a control treatment with the same amount of water (5 L at each application) added as the N addition treatments to 1×1.5 m plots. Five replicate plots of each treatment were established in five blocks used to control for microsite variation, with one plot of each treatment in each block. At the start of the field season in 2009, the plots were split in half, with one side randomly assigned as a recovery plot, receiving no further treatment solution, and the other side continuing to receive the treatment. No overland flow of treatment solution occurs between the subplots, and soils are relatively dry, and thus we assumed no belowground movement of the added N between treatment and recovery subplots.

Plant cover

We measured plant species composition and abundance in most years using a point-intercept approach. The upper 1×1 m portion of each plot was used for species composition measurements, one-half treatment and one-half recovery, and the bottom 1×0.5 m used for destructive soil sampling. Plant surveys were done in early July at the peak of the growing season when all species are present. Species were identified using Weber (1976) and Ackerfield (2015) with nomenclature following the USDA Plants database (*available online*).⁵

We used total plant cover as a coarse proxy for aboveground net primary productivity (NPP). Aboveground biomass, which is a good indicator of aboveground NPP in alpine dry meadows (Bowman et al. 2006), has been shown to correlate well with the cover of vascular plants in alpine communities (Ebert-May and Webber 1982).

Microbial abundance

Relative abundances of bacteria and fungi in soil from the control and 60 kg N·ha⁻¹·yr⁻¹ treatments, including treatment and recovery subplots, were quantified in 2014, after five years of recovery, using quantitative polymerase chain

⁵ <https://plants.usda.gov>

reaction (qPCR) approach (Fierer et al. 2005). Two surface soil (0–5 cm, A horizon) plugs were collected from each split plot using a sterile trowel and combined together to reduce the effect of in-plot variability. Samples were collected at three different times, representing early growing season (29 June), late growing season (13 August), and senescence (13 September) to assess the effects of seasonal variability. Bacterial (16S) and fungal (ITS) rRNA gene abundances were determined from 100-fold dilutions of genomic DNA extracted from 0.25 g of each bulk soil sample using a standard MoBio Soil DNA extraction kit (Carlsbad, California, USA). Genomic DNA was then amplified by running qPCR reactions in quadruplicate on 96-well qPCR plates. Standards for both 16S and ITS were made from plasmids containing full-length copies of the 16S and ITS rRNA gene from *Escherichia coli* K-12 and *Aspergillus fumigatus*, respectively. As standards, the concentration of genomic DNA from *E. coli* K-12 and *A. fumigatus* was assessed via a QuantiT-PicoGreen dsDNA assay (Invitrogen Life Technologies, Grand Island, New York, USA), and seven 10-fold dilutions were used to generate a standard curve, run in triplicate for each 96-well qPCR plate. Universal primer sets for the bacteria (515F, 806R) and fungi (FF390, FR1) were those described previously (Emerson et al. 2015). Each 20- μ L qPCR reaction well contained 12.5 μ L of ABgene SYBR Master Mix (Rochester, New York, USA), 1.25 μ L of each 10 mmol/L forward and reverse primers, 5 μ L of DNA-free water, and 5 μ L of 1:100 sample genomic DNA. An Eppendorf Realplex 2 thermocycler was used to carry out the reactions at 94°C for 3 min followed by 40 cycles of 94°C for 30 s, 50°C for 45 s, and 72°C for 30 s. Gene copy numbers were generated with a regression equation relating the threshold (C_t) value for each assay to the known number of copies in the standards as described in Emerson et al. (2015) with abundances reported as genome equivalents.

Soil chemistry

Soil solution NO_3^- concentrations were measured in 2010 and 2013 through 2017 at the start of the growing season prior to the application of the first aliquot of treatment solution using soil microlysimeters (Rhizon soil moist samplers, Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) inserted to a 5–7 cm depth. Soil moisture in 2011 and 2012 was too dry at the start of the growing season to obtain sufficient soil solution volumes for analysis. Two microlysimeters were used in each split plot. The soil solutions were analyzed for NO_3^- -N using a Dionex DX 500 System IonPac AS11 Ion Chromatograph (Sunnyvale, California, USA). Potential net N mineralization and nitrification were measured in 2012 and 2016. A single soil core (3 cm diameter \times 10 cm depth, A horizon) was collected from each split plot, placed in plastic bags, and brought back to the lab on ice, where it was divided into two vertical subsamples preserving the soil structure. One subsample was used for immediate analysis of extractable NH_4^+ -N and NO_3^- -N, and the other was placed in an incubator for 41 (2012) and 38 d (2016) at 12°C. The soils used for the 2012 incubation were maintained in open cups, and water was added to maintain constant soil moisture. The soils in the 2016 incubation were kept in gas-permeable plastic bags. Prior to analysis, the soils were sieved to 2 mm to remove rocks and

coarse organic material, shaken in 2 mol/L KCl for 1 h, then filtered. The filtrate was analyzed for NH_4^+ -N using a Lachat QuikChem 8000 Spectrophotometric Flow Injection Analyzer (Loveland, Colorado, USA), and NO_3^- -N was analyzed as described above for the soil water analysis. Net N mineralization and nitrification were calculated as the differences in total inorganic N and NO_3^- -N, respectively, between the two time periods. Subsamples of the sieved soils collected from the control and 60 kg N·ha⁻¹·yr⁻¹ plots for the 2016 incubations were analyzed for total carbon (C) and N after grinding the soil to fine powder and oven drying at 105°C to constant mass. The soils were analyzed using a Thermo Finnigan FLASH EA 1112 Series CHN Analyzer (Waltham, Massachusetts, USA).

As an additional estimate of the net production of NO_3^- -N in the soil during the growing season, one ion exchange resin bag per split plot was inserted below an undisturbed soil column at a 15 cm depth. The resin bags were constructed by inserting mixed-bed ion exchange resins (J. T. Baker, IONAC NM-60 H⁺/OH⁻; Phillipsburg, New Jersey, USA) into 1.25-cm³ plastic cylinders covered by nylon mesh. The resin bags were deployed in mid June 2016, prior to the first application of treatment solution to the plots, and retrieved after 37 d, after the first two applications of treatment solution were added to the plots. The beads were extracted with 2 mol/L KCl and analyzed for NO_3^- -N as previously described.

Soil extractable base cations and pH were measured in 2012 and 2016. Soil cores were collected from each split plot, sieved, and extracted using 0.1 mol/L BaCl₂. Exchangeable cations were analyzed using an ARL 3410 Inductively Coupled Plasma Emission Spectrophotometer (ICP-AES; Thermo Electron, Waltham, Massachusetts, USA). Soil pH was measured using a 1:2 soil:deionized water slurry with a Beckman 340 pH probe (Brea, California, USA).

Statistical analyses

Where measurements of response variables were made more than twice (plant species abundances, bacterial and fungi abundances, soil solution NO_3^-) the data were analyzed using repeated-measures multivariate analysis of variance, with N treatment level, plot type (treatment cessation vs. continued treatment), and block as model effects. Linear regressions were also used to assess within year effects of the N treatment on soil NO_3^- concentrations. Net N mineralization and nitrification, soil %N, C:N, and pH, and soil extractable cations were analyzed within each year using two-way analysis of variance, with N treatment, plot type, and block as model effects. Differences in plant species composition among the treatments were tested using permutational multivariate ANOVA (Anderson 2001) on a Bray-Curtis dissimilarity matrix and visualized with nonmetric multidimensional scaling using the R package vegan (version 2.4-3; Oksanen et al. 2017) in R version 3.4.0 (R Core Team 2017).

RESULTS

Plant responses

Previously, we reported that simulated N deposition increased the cover of the sedge *Carex rupestris* in a dose-

dependent manner within 4 yr of treatment initiation (Bowman et al. 2006) and found that after 2005, the eighth year of the experiment, the cover of the dominant plant species of the dry meadow community *Kobresia myosuroides* decreased significantly, also in a dose-dependent manner. Following cessation of N additions to treated plots there was a decrease in *Carex* cover in the recovery plots (Fig. 1a; repeated-measures ANOVA, time \times plot type; $F_{6,27} = 2.79$, $P = 0.03$), which was most apparent in the 40 kg N·ha⁻¹·yr⁻¹ plots. The cover of *Kobresia* did not change significantly in recovery plots (Fig. 1b; repeated-measures ANOVA, time \times plot type; $F_{6,27} = 1.67$, $P = 0.17$).

Community composition in the N addition plots diverged from the control plots within 4 yr of the start of the experiment (1997–2000; Bowman et al. 2006). Over the eight years following cessation of the N treatment additions, there was no indication of significant recovery of community composition based on repeated-measures PERMANOVA (time \times N treatment \times plot type, $F_{3,27} = 0.494$, $P = 0.41$). While there were significant changes in community composition with time in all treatments (time, $F_{1,27} = 4.77$, $P < 0.001$), the changes were not directional in any of the plots (Fig. 2) with the exception of the 40 kg N·ha⁻¹·yr⁻¹ recovery plots, which trended toward the centroids of control plots. Much of the interannual variability may reflect changes in weather or population processes that influenced the abundances of some species (Spasojevic et al. 2013).

Total plant cover, used as an estimate of aboveground NPP, showed a slight overall decline with time (8% over 7 yr, data not shown; repeated measures ANOVA, time; $F_{6,30} = 5.47$, $P < 0.001$) with lower cover in plots receiving higher N inputs (4% decrease at the highest N input; $F_{1,35} = 4.43$, $P = 0.042$). There was no time \times N treatment interaction ($F_{6,30} = 1.18$, $P = 0.31$) nor a time \times N treatment \times plot type interaction ($F_{6,30} = 0.26$, $P = 0.95$). Thus, estimated NPP was slightly lower in the high N treatment plots, and was not affected by ending the N additions in the recovery plots.

Microbial responses

To evaluate coarse level changes in microbial community composition, we measured bacterial and fungal abundances (16S and ITS rRNA gene counts, respectively) in the control and 60 kg N·ha⁻¹·yr⁻¹ treatment and recovery plots, five years after ending application of the treatments. Since microbial biomass and community composition can change seasonally (Lipson and Schmidt 2004, Bardgett et al. 2005), we measured microbial abundances during the early and middle parts of the growing season and after plant senescence. Both fungal and bacterial abundances decreased in response to the N treatment ($F_{1,68} = 7.69$, $P = 0.007$ for the fungal abundance, $F_{1,68} = 25.88$, $P < 0.001$ for bacterial abundance, Fig. 3). There was no indication of recovery of bacterial and fungal abundances in the recovery plots (data not shown, N treatment \times plot type; $F_{1,68} = 2.10$, $P = 0.15$ for fungal abundance, $F_{1,68} = 1.57$, $P = 0.21$ for bacterial abundance). Fungal abundances changed seasonally, but only in the N treatment plots (time \times N treatment; $F_{2,67} = 3.31$, $P = 0.05$). There was no significant seasonal change in bacterial abundance (time effect; $F_{2,67} = 1.71$, $P = 0.19$; time \times N treatment; $F_{2,67} = 0.14$, $P = 0.87$).

Soil responses

In most years soil solution NO₃⁻ concentrations measured at the start of the growing season (prior to the addition of treatment solutions) were elevated in the N treatment plots relative to the control plots (repeated measures, N treatment, $F_{1,7} = 8.40$, $P = 0.02$), though there was no trend in the concentrations through time (time; $F_{4,4} = 0.64$, $P = 0.64$) nor an effect of plot type on temporal trends (time \times plot type; $F_{4,4} = 0.18$; $P = 0.94$). For some years (2010 and 2014) neither the treatment nor the recovery plots showed significant correlations of soil solution NO₃⁻ concentrations with N treatment. In 2013 and 2016, both treatment and recovery

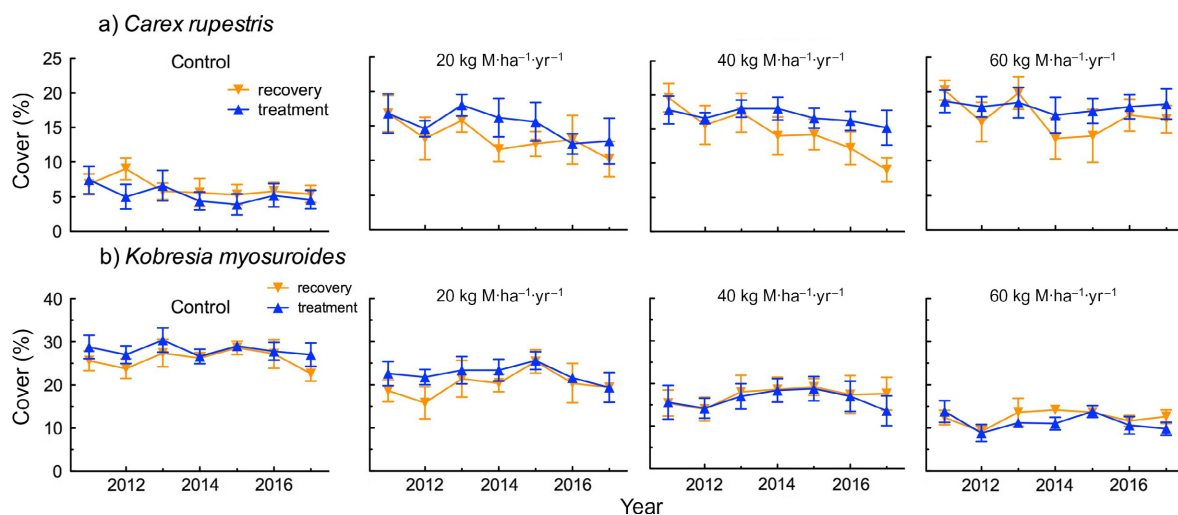


FIG. 1. Projected cover of the nitrophilic species *Carex rupestris* (a) and the dominant sedge of the dry meadow community *Kobresia myosuroides* (b) in plots receiving 0, 20, 40, or 60 kg N·ha⁻¹·yr⁻¹ since 1997 (treatment, blue symbols), and plots that received the same dosage between 1997 and 2008, but then received no treatment since 2009 (recovery, orange symbols). Symbols are means ($n = 5$), error bars show \pm SE.

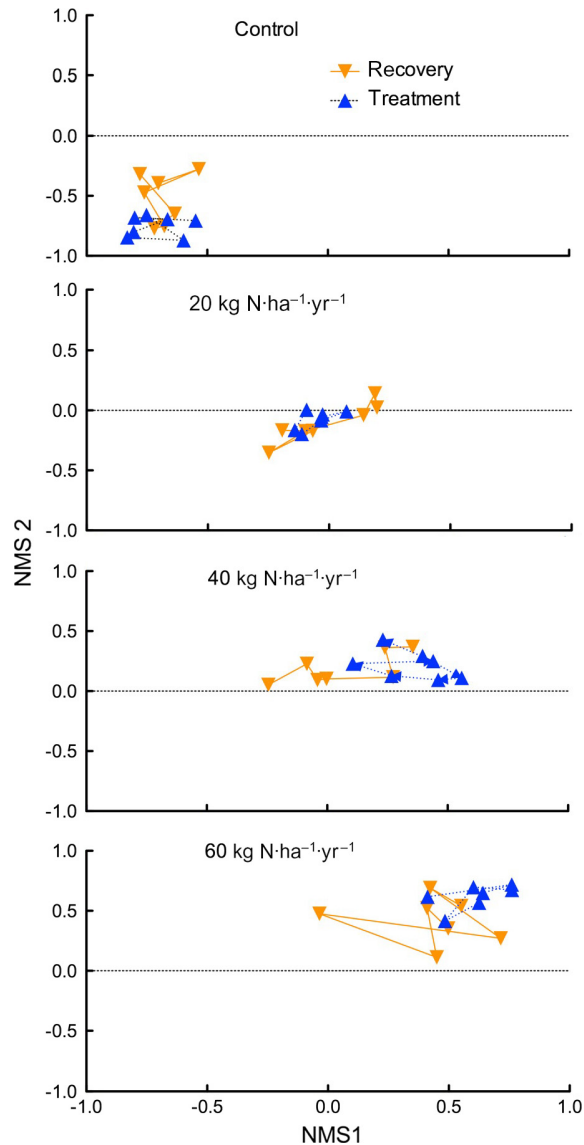


FIG. 2. Nonmetric multidimensional scaling (NMS) ordination analysis showing interannual trends in plant species composition for recovery (orange symbols) and treatment (blue symbols) plots in 0, 20, 40, and 60 kg N·ha⁻¹·yr⁻¹ treatments. The symbols are means ($n = 5$) and connecting lines note the direction of change between years.

plots showed significant positive correlations between soil solution NO_3^- concentrations and N treatment (Fig. 4). In 2017 only the treatment plots showed a significant correlation with N treatment, although the soil solution NO_3^- concentrations were elevated in the intermediate N treatments in recovery plots (Fig. 4). Resin bag extractable NO_3^- measured in 2016 was also elevated in both treatment and recovery plots (Fig. 5; $F_{1,37} = 17.4$, $P < 0.001$) with higher amounts in the treatment relative to the recovery plots (N treatment \times plot type, $F_{1,37} = 4.30$, $P = 0.05$). Net N mineralization in the incubated soils was not significantly affected by the N treatments in either 2012 or 2016 (data not shown, mean rate -0.42 mg N·kg soil⁻¹·d⁻¹, $F_{1,37} = 2.83$, $P = 0.10$ for 2012; mean rate = 0.36 mg N·kg soil⁻¹·d⁻¹; $F_{1,37} = 0.03$, $P = 0.85$; treatment \times recovery, $F_{7,32} = 0.09$, $P = 0.76$), the same result found earlier after 7 yr of treatment (Bowman et al. 2006).

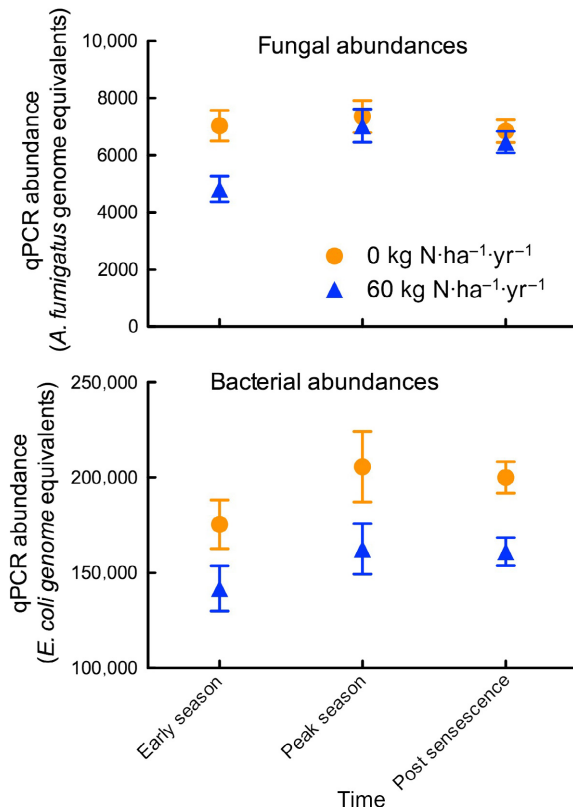


FIG. 3. Seasonal trends in bacterial and fungal abundances in plots receiving 0 (orange circles) and 60 kg N·ha⁻¹·yr⁻¹ (blue triangles). No differences were found between treatment and recovery plots. Data are reported as genome equivalents. Symbols are means ($n = 5$), error bars show \pm SE.

Net nitrification rates were significantly influenced by N treatment in 2016, but not in 2012 (Fig. 6; $F_{1,37} = 0.19$, $P = 0.66$ for 2012; $F_{1,37} = 3.73$, $P = 0.02$ for 2016). Nitrification rates were not affected by cessation of the N treatment, with recovery plots not differing from treatment plots for the 2016 measurements (N treatment \times plot type, $F_{3,68} = 0.004$, $P = 0.95$). Soil C:N ratios were not different between the control and 60 kg N·ha⁻¹·yr⁻¹ treatments nor was there a significant treatment \times plot type interaction (13.80 ± 0.31 for control, 13.75 ± 0.20 for the 60 kg N·ha⁻¹·yr⁻¹ treatment; $F_{1,15} = 0.03$, $P = 0.85$ for treatment effect, $F_{1,15} = 0.10$, $P = 0.76$ for treatment \times plot type interaction). There was a significant treatment effect on soil N concentration (%N: 0.083 ± 0.005 for control, 0.101 ± 0.007 for the 60 kg N·ha⁻¹·yr⁻¹ treatment; $F_{1,15} = 4.85$, $P = 0.05$) but no treatment \times plot type interaction ($F_{1,15} = 0.001$, $P = 0.97$).

We previously reported that after 11 yr of simulated N deposition (2008) the availability of the nutrient base cation magnesium (soil extractable Mg^{2+}) and soil pH decreased, while the amount of the cations Al^{3+} and Mn^{2+} increased (Lieb et al. 2011). Similar results were found for measurements made in 2012 and 2016 (Figs. 7 and 8, Table 1). Extractable Mg^{2+} was lower in the highest N treatments in both 2012 and 2016, and there was a trend ($P = 0.08$) toward lower Ca^{2+} in N treatment plots in 2012. Conversely, Mn^{2+} and Al^{3+} concentrations increased with N inputs in both years. Soil pH was lower in N-treated plots in both

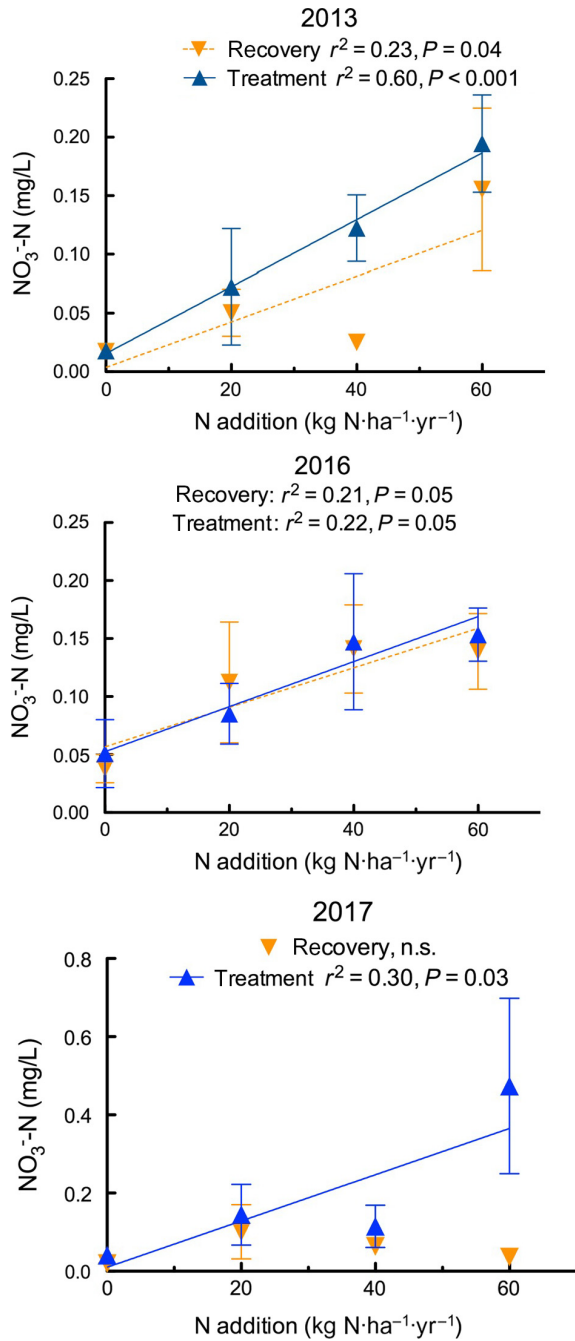


FIG. 4. Soil solution NO_3^- concentrations for recovery (orange symbols, dotted lines) and treatment (blue symbols, solid lines) plots receiving 0, 20, 40, or 60 $\text{kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ for 2013, 2016, and 2017. Samples were collected prior to the application of the treatment solutions for the year in the treatment plots. Symbols are means ($n = 5$), error bars show \pm SE. Note the different y-axis scales between 2017 and the previous years. No significant (n.s.) increase in soil solution NO_3^- concentrations was found in relation to the treatment levels in 2010 and 2014.

years. None of these responses to N deposition showed any trends toward reversal in the recovery plots (no significant N treatment \times plot type interactions), with the exception of Mg^{2+} in the 60 $\text{kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ plots, which was higher in recovery plots than treatment plots in 2016 (Fig. 7).

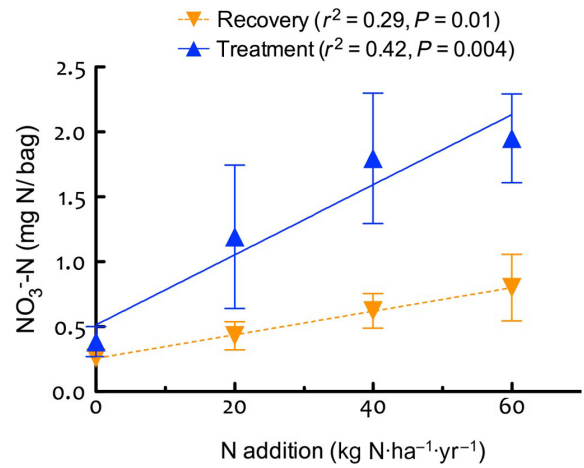


FIG. 5. NO_3^- extracted from resins in bags buried in the soil during the growing season in 2016 in treatment plots (blue symbols, solid lines) subjected to 0, 20, 40, or 60 $\text{kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ and recovery plots (orange symbols, dotted lines). Symbols are means ($n = 5$), error bars show \pm SE.

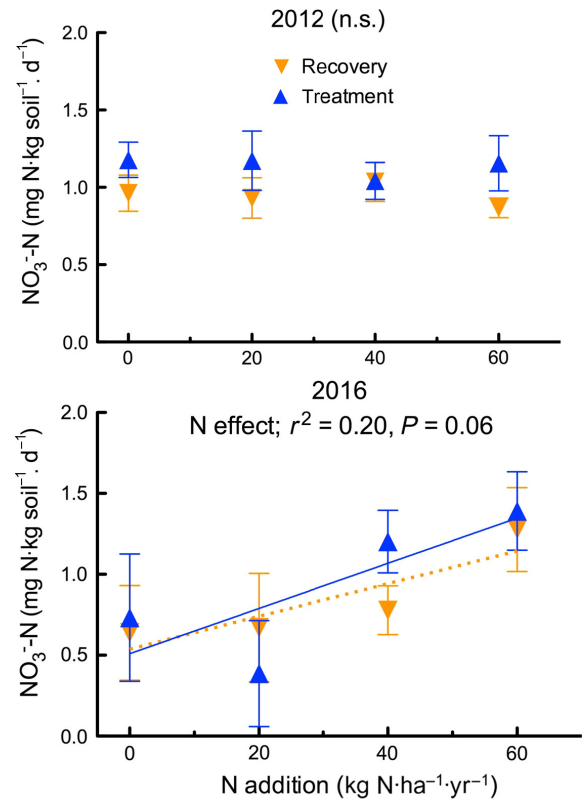


FIG. 6. Potential nitrification rates for soils analyzed in 2012 and 2016 in treatment plots (blue symbols, solid lines) receiving 0, 20, 40, or 60 $\text{kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ and recovery plots (orange symbols, dotted lines) no longer receiving the treatments. Symbols are means ($n = 5$), error bars show \pm SE.

DISCUSSION

There is abundant evidence for the potential and realized impacts of N deposition in terrestrial ecosystems. Losses of plant diversity and eutrophication are widespread in

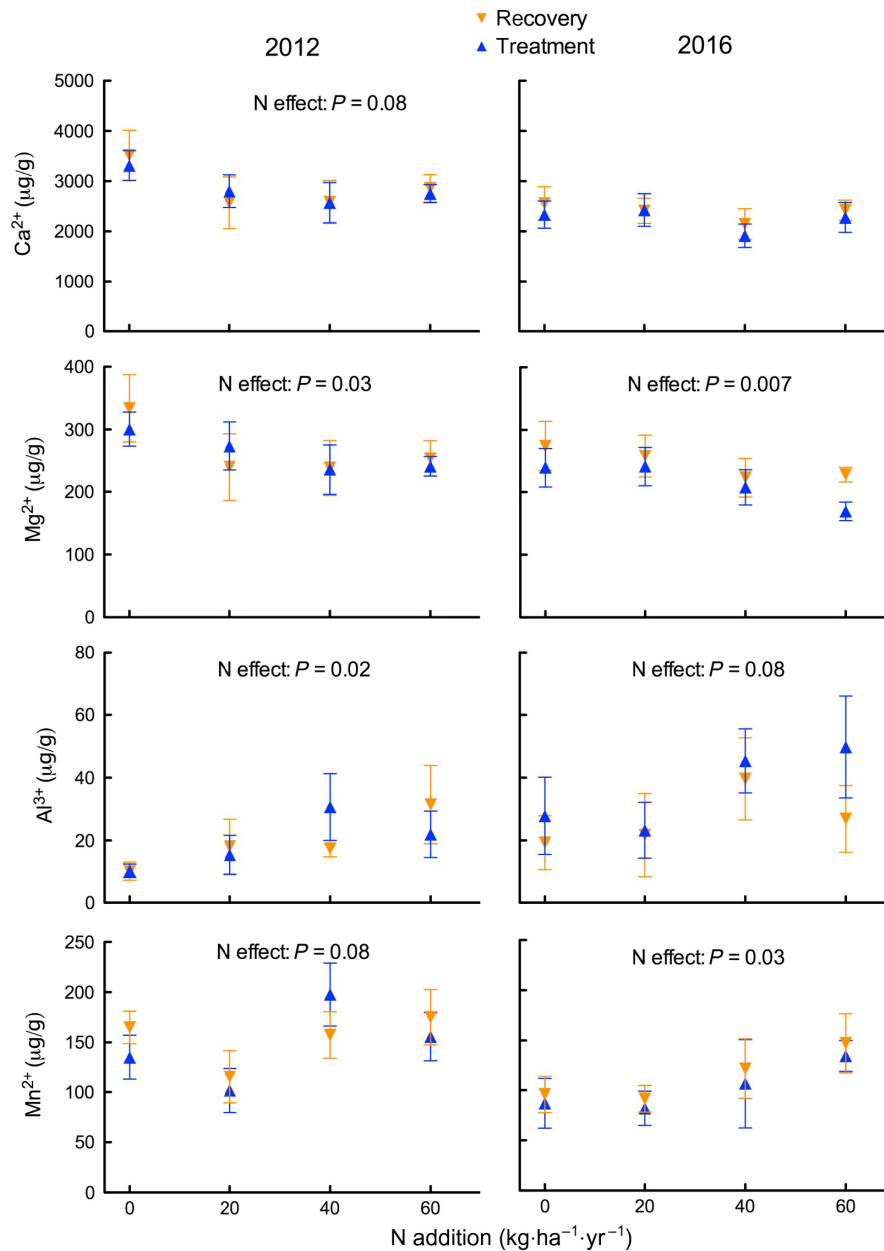


FIG. 7. Soil extractable cations collected and analyzed in 2012 and 2016 from treatment plots (blue symbols) receiving 0, 20, 40, or 60 kg N·ha⁻¹·yr⁻¹ and recovery plots (orange symbols) no longer receiving the treatments. Symbols are means ($n = 5$), error bars show \pm SE. Statistical analyses are described in Table 1.

developed regions (Stevens et al. 2004, Bobbink et al. 2010, Simkin et al. 2016, Clark et al. 2017) and acidification of soils and surface waters have occurred in the most heavily impacted regions (Driscoll et al. 2001, Bowman et al. 2008, Tian and Niu 2015). It is unclear whether ecosystems can recover from N deposition impacts as emissions of reactive N are lowered with air quality improvements is uncertain as there are biogeochemical feedbacks that may limit the return of community and soil chemistry to pre-impact conditions (Groffman et al. 2006, Stevens 2016). Maintenance of elevated soil N cycling rates, occupancy within the community by nitrophilic species, and very slow weathering rates may limit biological and geochemical recovery of ecosystems after reductions in N deposition rates.

Here, we investigated the changes in microbial and plant communities, N cycling, and base cation chemistry in an alpine dry meadow for nine years following cessation of a 12-yr N deposition experiment (Bowman et al. 2006, Lieb et al. 2011). Based on conceptual models of ecosystem responses to N deposition derived from experimental and observational research (Aber et al. 1998, Lovett and Goodale 2011, Bowman et al. 2015, Clark et al. 2017), we hypothesized that plant and microbial communities, which typically respond early to N deposition, would show the greatest recovery relative to soil geochemical impacts, including the loss of base cations, increase in metal cations, and lower soil pH. As predicted, the changes in plant species abundances showed some recovery, mainly in recovery plots that had received

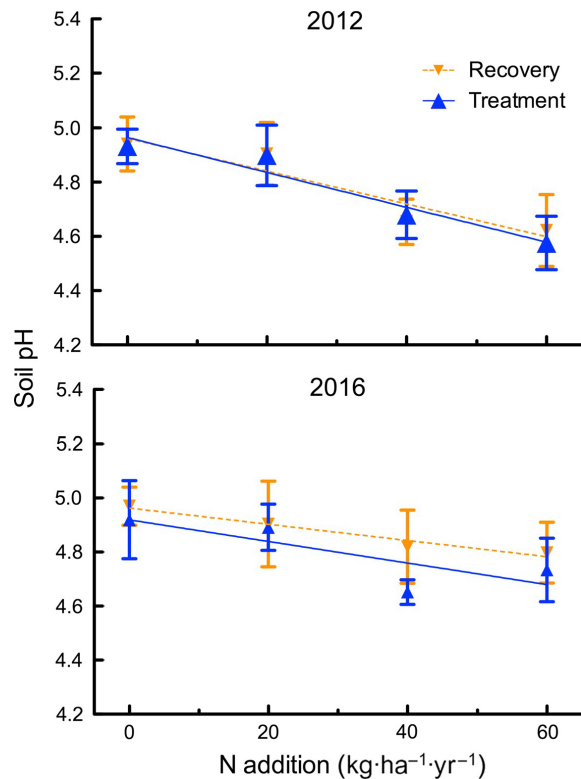


FIG. 8. Soil pH for treatment plots (blue symbols, solid lines) receiving 0, 20, 40, or 60 kg N·ha⁻¹·yr⁻¹ and recovery plots (orange symbols, dotted lines) no longer receiving the treatments collected and analyzed in 2016. Symbols are means (n = 5), error bars show ±SE.

TABLE 1. ANOVA results for the effects of N treatment, plot type (recovery, treatment), and the N treatment × plot type interaction on variation of extractable cations and pH in soils collected in 2012 and 2016.

Parameter	N treatment		Plot type		N treatment × Plot type	
	F	P	F	P	F	P
Cation by year						
2012						
Ca ²⁺	3.23	0.08	0.10	0.92	0.0004	0.98
Mg ²⁺	5.36	0.03	0.03	0.87	0.01	0.90
K ⁺	2.64	0.11	0.07	0.79	0.09	0.76
Mn ²⁺	3.17	0.08	4.18	0.05	0.85	0.36
Al ³⁺	6.06	0.02	2.90	0.10	1.36	0.25
pH	20.5	<0.001	0.02	0.89	0.14	0.89
2016						
Ca ²⁺	0.75	0.39	0.68	0.41	0.00	0.99
Mg ²⁺	8.21	0.007	3.71	0.06	0.23	0.63
K ⁺	1.06	0.31	1.67	0.21	0.50	0.48
Mn ²⁺	4.97	0.03	0.42	0.52	0.01	0.92
Al ³⁺	3.23	0.08	1.46	0.23	0.44	0.51
pH	4.52	0.04	1.00	0.34	0.09	0.76

intermediate levels of N. In contrast there was no evidence of a recovery in bacterial and fungal abundances, which is surprising given their more rapid turnover and high sensitivity to soluble soil N (Treseder 2008, Ramirez et al. 2012). N cycling rates and pools of soil NO₃⁻ and total N remained

elevated in recovery plots, and there was no indication of changes in soil cation levels or soil pH during the eight years following cessation of the N treatments. Relatively high ambient rates of N deposition at our research site (total 6 kg N·ha⁻¹·yr⁻¹), which exceeds the critical load for plant and algal species composition and dissolved inorganic N in unvegetated talus slopes (Williams and Tonnesen 2000, Baron 2006, Bowman et al. 2006) may also have contributed to the lack of recovery. Our results are consistent with previous work indicating that the recovery of ecosystems from N deposition impacts is slow (reviewed in Stevens 2016), but extends our understanding by evaluating the metrics that are expected to recover earliest and the underlying factors slowing ecosystem recovery. These results contribute guidance in determining the factors that should be used for determining management standards (e.g., critical loads for pollutants), such as indicator species that respond early prior to changes in less reversible factors such as soil chemistry.

The recovery of the dry meadow plant community from the impacts of N deposition was marked by a decrease in the cover of the nitrophilic species, *Carex rupestris*, which had increased cover by around three-fold with simulated N deposition, from 5% to 15% of the cover. The reversal of the enhanced cover was most pronounced in the 40 kg N·ha⁻¹·yr⁻¹ plots and not apparent in the 60 kg N·ha⁻¹·yr⁻¹ plots, and determined the community responses to cessation of N addition seen in Fig. 2. This result is consistent with our hypothesis that the recovery of vegetation would be less pronounced or absent in recovery plots that had previously received the highest N inputs. The decrease in the cover of the dominant species *Kobresia myosuroides* (from around 25% to 10% in the 60 kg N·ha⁻¹·yr⁻¹ plots) was unaffected nine years after N inputs ceased. The continued depression in *Kobresia* cover may be linked with decreases in mycorrhizal abundance (T. S. Potter, W. M. Owens, and W. D. Bowman, unpublished manuscript) or possibly changes in soil geochemistry (loss of nutrient cations and/or increase in toxic cations). Generally species recovery from the impacts of N deposition are individualistic (Stevens 2016), with some species recovering (e.g., legumes; Storkey et al. 2015) and others showing limited responses (e.g., mosses; Emmett 2007). Fast-growing, short-lived species are more likely to exhibit rapid changes in population growth and respond positively to N inputs than slow-growing long-lived species. Thus, nitrophilic species may exhibit greater change (decreases in abundance) following decreases in N deposition than species that decline as a result of N deposition. Differing species' responses to lower N deposition results in variable changes in diversity among different studies (Stevens 2016), emphasizing the merits of monitoring individual species responses as shown here.

Nitrogen fertilization generally lowers the abundances of bacteria and fungi in soils (Wallenstein et al. 2006, Nemergut et al. 2008, Treseder 2008, Ramirez et al. 2012), and we found that both fungal and bacterial abundances decreased by around 20% with simulated N deposition, similar to the decreases reported in other studies (15–30% for total microbial biomass). However, there was no indication that either bacterial or fungal abundances recovered following cessation of the N inputs. The apparent effects of the simulated N deposition on fungal abundances changed seasonally, with significant decreases only during the early part of the growing

season, but not at peak season or after plant senescence when labile carbon tends to be more available in alpine soils (Schmidt and Lipson 2004). Bacterial abundances remained lower in the N treatment throughout the growing season. The overall decrease in microbial abundances may ultimately lower the capacity of these soils to immobilize reactive N via uptake and incorporation into microbial biomass (Treseder 2008). However, the slower turnover of fungal biomass results in better long-term sequestration (Bardgett et al. 2005, Högborg et al. 2013), although the lower fungal abundance of the N treated plots in the early part of the growing season corresponds to the period of highest N leaching when the need for stabilization is greatest.

The pools of soil solution and resin bag NO_3^- remained elevated in the recovery plots relative to controls, likely resulting in part from elevated rates of nitrification. Soil N concentrations also remained elevated in recovery plots, although soil C:N did not differ between the control and highest N treatment. Additionally, as noted above, the uptake of N into microbial and plant biomass may have decreased, contributing to higher pools of soluble N, although microbial and plant N uptake were not measured. An increase in bacterial and archaeal nitrifiers may have contributed to higher nitrification rates (He et al. 2007), although an earlier study did not find an increase in nitrifiers with N fertilization in alpine soils (Nemergut et al. 2008). Elevated N cycling rates have been reported in plots decades after N fertilization has stopped in grasslands (e.g., Milchunas and Lauenroth 1995, Clark et al. 2009). A survey of previous studies indicates that pools of inorganic N in soils often recover quickly following reduction in N deposition (Stevens 2016). The lack of a eutrophying effect of N deposition in alpine dry meadows, as shown by a small decrease in total plant cover in this experiment, and weak or no increases NPP in the first 4 yr (Bowman et al. 2006) may in part explain the difference between our findings and those described in Stevens (2016), which included systems with greater biotic sinks (higher plant growth rates) for labile N.

The most severe impacts of N deposition are associated with losses of base cations, acidification of soils and surface waters, and increased levels of toxic metal cations (Aber et al. 1998, Bowman et al. 2015, 2008). As long as NO_3^- production outstrips biotic uptake or physical sequestration in soil, the resulting increases in soil solution NO_3^- concentrations will continue to enhance leaching of base cations. Relatively slow weathering rates may not keep pace with base cation losses, lowering soil fertility and pH (Bowman et al. 2008). At the highest inputs of N soil geochemical responses in alpine dry meadows were marked by lower Mg and soil pH, and higher Al and Mn, all of which may be detrimental for alpine biota (Tian et al. 2016). Surprisingly, the decrease in *Kobresia* cover was not directly associated with Al toxicity, which along with *Carex rupestris* exhibits robust tolerance to soluble Al in the soil (T. S. Potter, W. M. Owens, and W. D. Bowman, *unpublished manuscript*). As hypothesized, the recovery of soil cations and pH did not occur in the eight years following the halt in N additions, with the exception of Mg in the $60 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ treatment. This result may reflect a decrease in Mg in the treatment plots based on the comparison with 2012 results. In contrast to our finding, Högborg et al. (2006) found significant recoveries of base

cations and soil pH a decade after cessation of high N inputs ($108 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$) in a boreal forest. The continued elevation of nitrification rates observed in our experiment in the recovery plots and subsequent elevated amount of NO_3^- in soil solution will hinder recovery of cations and soil pH. One twist in this story is the supply of cations from dust deposition, which contributes a substantial amount of Ca and Mg to alpine soils in the Front Range (Litaor 1987, Muhs and Benedict 2006). Dust deposition has been increasing over the past several decades (Brahney et al. 2013), and may be preventing even greater losses of soil buffering capacity associated with N deposition in our plots. Measurement of strontium isotopes (as a proxy for Ca) indicates nearly all of the Ca in plants is derived from dust deposition rather than from bedrock weathering at our research site (W. D. Bowman and J. C. Neff, *unpublished data*).

In summary, we found that most of the impacts associated with 12 yr of simulated N deposition were sustained during the 9 yr following halting the N addition treatments, with little indication of recovery. Only the cover of the nitrophilic species *Carex rupestris* decreased in association with cessation of N additions. Nitrification rates and pools of NO_3^- remained the same in recovery and treatment plots, which probably contributed to the lack of recovery of soil cations and pH. The trajectory of recovery of these soil geochemical factors will likely be slow, although potentially enhanced by dust deposition bringing in exogenous base cations. Given the potential for long-lasting impacts of N deposition on ecosystem structure and function, including the decline of key ecosystem services such as water quality and forage (Galloway et al. 2008, Clark et al. 2017) selecting metrics that show greater recovery such as species abundances as critical load standards should be considered to prevent more severe impacts.

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LITERATURE CITED

- Aber, J., W. McDowell, K. Nadelhoffer, A. Magill, G. Berntson, M. Kamakea, S. McNulty, W. Currie, L. Rustad, and I. Fernandez. 1998. Nitrogen saturation in temperate forest ecosystems—hypotheses revisited. *BioScience* 48:921–934.
- Ackerfield, J. 2015. *Flora of Colorado*. Botanical Research Institute of Texas, Fort Worth, Texas, USA.
- Anderson, M. J. 2001. A new metric for non-parametric multivariate analysis of variance. *Austral Ecology* 26:32–46.
- Bardgett, R. D., W. D. Bowman, R. Kaufmann, and S. K. Schmidt. 2005. A temporal approach to linking aboveground and belowground ecology. *Trends in Ecology and Evolution* 20:634–641.
- Baron, J. S. 2006. Hindcasting nitrogen deposition to determine an ecological critical load. *Ecological Applications* 16:433–439.
- Bobbink, R., et al. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications* 20:30–59.

- Bowman, W. D., and H. Steltzer. 1998. Positive feedbacks to anthropogenic nitrogen deposition in Rocky Mountain alpine tundra. *Ambio* 27:514–517.
- Bowman, W. D., J. R. Gartner, K. Holland, and M. Wiedermann. 2006. Nitrogen critical loads for alpine vegetation and terrestrial ecosystem response: are we there yet? *Ecological Applications* 16:1183–1193.
- Bowman, W. D., C. C. Cleveland, L. Halada, J. Hreško, and J. S. Baron. 2008. Negative impact of nitrogen deposition on soil buffering capacity. *Nature Geoscience* 1:767–770.
- Bowman, W. D., D. R. Nemergut, D. M. McKnight, M. P. Miller, and M. W. Williams. 2015. A slide down a slippery slope—alpine ecosystem responses to nitrogen deposition. *Plant Ecology and Diversity* 8:597–605.
- Boxman, A. W., P. J. M. van der Ven, and J. G. M. Roelofs. 1998. Ecosystem recovery after a decrease in nitrogen input to a Scots pine stand Ysselsteyn, the Netherlands. *Forest Ecology and Management* 101:155–163.
- Brahney, J., A. P. Ballantyne, C. Sievers, and J. C. Neff. 2013. Increasing Ca^{2+} deposition in the western US: the role of mineral aerosols. *Aeolian Research* 10:77–87.
- Choma, M., M. O. Rappe-George, J. Bárta, P. Čapek, E. Kaštovská, A. I. Gärdenäs, and H. Šantrůčková. 2017. Recovery of the ectomycorrhizal community after termination of long-term nitrogen fertilisation of a boreal Norway spruce forest. *Fungal Ecology* 29:116–122.
- Clark, C. M., S. E. Hobbie, R. Venterea, and D. Tilman. 2009. Long-lasting effects on nitrogen cycling 12 years after treatments cease despite minimal long-term nitrogen retention. *Global Change Biology* 15:1755–1766.
- Clark, C. M., M. D. Bell, J. W. Boyd, J. E. Compton, E. A. Davidson, C. Davis, M. E. Fenn, L. Geiser, L. Jones, and T. F. Blett. 2017. Nitrogen-induced terrestrial eutrophication: cascading effects and impacts on ecosystem services. *Ecosphere* 8:e01877.
- Driscoll, C. T., G. B. Lawrence, A. J. Bulger, T. J. Butler, C. S. Cronan, C. Eagar, K. F. Lambert, G. E. Likens, J. L. Stoddard, and K. C. Weathers. 2001. Acidic deposition in the northeastern United States: Sources and inputs, ecosystem effects, and management strategies. *BioScience* 51:180–198.
- Du, E., W. de Vries, J. N. Galloway, X. Hu, and J. Fang. 2014. Changes in wet nitrogen deposition in the United States between 1985 and 2012. *Environmental Research Letters* 9:095004.
- Ebert-May, D., and P. J. Webber. 1982. Spatial and temporal variation of vegetation and its productivity on Niwot Ridge, Colorado. Pages 35–62 in J. Halfpenny, editor. *Ecological studies in the Colorado alpine*, a festschrift for John W. Marr (Occasional paper number 37). Institute of Arctic and Alpine Research, University of Colorado, Boulder, Colorado, USA.
- Emerson, J. B., P. B. Keedy, T. E. Brewer, N. Clements, E. E. Morgan, J. Awerbuch, S. L. Miller, and N. Fierer. 2015. Impacts of flood damage on airborne bacteria and fungi in homes after the 2013 Colorado Front Range flood. *Environmental Science & Technology* 49:2675–2684.
- Emmett, B. 2007. Nitrogen saturation of terrestrial ecosystems: some recent findings and their implications for our conceptual framework. *Water Air and Soil Pollution: Focus* 7:99–109.
- Fierer, N., J. A. Jackson, R. Vilgalys, and R. B. Jackson. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiology* 71:4117–4120.
- Freedman, Z. A., R. A. Upchurch, and D. R. Zak. 2016. Microbial potential for ecosystem N loss is increased by experimental N deposition. *PLoS ONE* 11(10):e0164531.
- Galloway, J. N., A. R. Townsend, J. W. Erisman, M. Bekunda, Z. Cai, J. R. Freney, L. A. Martinelli, S. P. Seitzinger, and M. A. Sutton. 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320:889–892.
- Groffman, P. M., et al. 2006. Ecological thresholds: the key to successful environmental management or an important concept with no practical application? *Ecosystems* 9:1–13.
- Harris, D. J., K. G. Smith, and P. J. Hanly. 2011. Occupancy is nine-tenths of the law: occupancy rates determine the homogenizing and differentiating effects of exotic species. *American Naturalist* 177:535–543.
- He, J., J. Shen, L. Zhang, Y. Zhu, Y. Zheng, M. Xu, and H. J. Di. 2007. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. *Environmental Microbiology* 9:2364–2374.
- Högberg, M. N., L. Hogbom, and D. B. Kleja. 2013. Soil microbial community indices as predictors of soil solution chemistry and N leaching in *Picea abies* (L.) Karst. Forests in S. Sweden. *Plant and Soil* 372:507–522.
- Högberg, P., F. Houbau, M. Quist, D. Binkley, and C. O. Tamm. 2006. Tree growth and soil acidification in response to 30 years of experimental nitrogen loading on boreal forest. *Global Change Biology* 12:489–499.
- Lieb, A. M., A. Darrouzet-Nardi, and W. D. Bowman. 2011. Nitrogen deposition decreases acid buffering capacity of alpine soils in the southern Rocky Mountains. *Geoderma* 164:220–224.
- Lipson, D. S., and S. K. Schmidt. 2004. Seasonal changes in an alpine soil bacterial community in the Colorado Rocky Mountains. *Applied and Environmental Microbiology* 70:2867–2879.
- Litaor, M. I. 1987. The influence of eolian dust on the genesis of alpine soils in the Front Range, Colorado. *Soil Science Society of America Journal* 51:142–147.
- Liu, X., et al. 2013. Enhanced nitrogen deposition over China. *Nature* 494:459–462.
- Lovett, G. M., and C. L. Goodale. 2011. A new conceptual model of nitrogen saturation based on experimental nitrogen addition to an oak forest. *Ecosystems* 14:615–631.
- Milchunas, D. G., and W. K. Lauenroth. 1995. Inertia in plant community structure: state changes after cessation of nutrient enrichment stress. *Oecologia* 5:452–458.
- Muhs, D. R., and J. B. Benedict. 2006. Eolian additions to late Quaternary alpine soils, Indian Peaks Wilderness Area, Colorado Front Range. *Arctic Antarctic and Alpine Research* 38:120–130.
- Neff, J. C., A. P. Ballantyne, G. L. Farmer, N. M. Mahowald, J. L. Conroy, C. C. Landry, J. T. Overpeck, T. H. Painter, C. R. Lawrence, and R. L. Reynolds. 2008. Increasing eolian dust deposition in the western United States linked to human activity. *Nature Geoscience* 1:189–195.
- Nemergut, D. R., A. R. Townsend, S. R. Sattin, K. R. Freeman, N. Fierer, J. C. Neff, W. D. Bowman, C. W. Schadt, M. N. Weintraub, and S. K. Schmidt. 2008. The effects of chronic nitrogen fertilization on alpine tundra soil microbial communities: implications for carbon and nitrogen cycling. *Environmental Microbiology* 10:3093–3105.
- Nohrstedt, H.-Ö. 1998. Residual effects of N fertilization on soil-water chemistry and ground vegetation in a Swedish Scots pine forest. *Environmental Pollution* 102:77–83.
- Oksanen, J., et al. 2017. *vegan: Community Ecology Package*. R package version 2.4-3. <https://CRAN.R-project.org/package=vegan>
- R Core Team. 2017. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Ramirez, K. S., C. M. Craine, and N. Fierer. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology* 18:1918–1927.
- Schmidt, S. K., and D. A. Lipson. 2004. Microbial growth under the snow: implications for nutrient and allelochemical availability in temperate soils. *Plant and Soil* 259:1–7.
- Simkin, S. M., et al. 2016. Conditional vulnerability of plant diversity to atmospheric nitrogen deposition across the USA. *Proceedings of the National Academy of Sciences USA* 113:4086–4091.
- Spasojevic, M. J., W. D. Bowman, H. C. Humphries, T. R. Seastedt, and K. N. Suding. 2013. Changes in alpine vegetation over 21 years: are patterns across a heterogeneous landscape consistent with predictions? *Ecosphere* 4:art117.
- Stevens, C. J. 2016. How long do ecosystems take to recover from atmospheric nitrogen deposition? *Biological Conservation* 200:160–167.

- Stevens, C. J., N. B. Dise, J. O. Mountford, and D. J. Gowing. 2004. Impact of nitrogen deposition on the species richness of grasslands. *Science* 303:1876–1879.
- Storkey, J., A. J. Macdonald, P. R. Poulton, T. Scott, I. H. Köhler, H. Schnyder, K. W. T. Goulding, and M. J. Crawley. 2015. Grassland biodiversity bounces back from long-term nitrogen addition. *Nature* 58:401–404.
- Strengbom, J., A. Nordin, T. Näsholm, and L. Ericson. 2001. Slow recovery of boreal forest ecosystem following decreased nitrogen input. *Functional Ecology* 15:451–457.
- Thomas, R. Q., C. D. Canham, K. C. Weathers, and C. L. Goodale. 2010. Increased tree carbon storage in response to nitrogen deposition in the US. *Nature Geoscience* 3:13–17.
- Tian, D., and S. Niu. 2015. A global analysis of soil acidification caused by nitrogen addition. *Environmental Research Letters* 10:024019.
- Tian, Q. Y., et al. 2016. A novel soil manganese mechanism drives plant species loss with increased nitrogen deposition in a temperate steppe. *Ecology* 97:65–74.
- Treseder, K. K. 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology Letters* 11:1111–1120.
- van Breemen, N., J. Mulder, and C. T. Driscoll. 1983. Acidification and alkalization of soils. *Plant and Soil* 75:283–308.
- Vinton, M. A., and I. C. Burke. 1995. Interactions between individual plant species and soil nutrient status in shortgrass steppe. *Ecology* 76:1116–1133.
- Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* 7:737–750.
- Waldner, P., et al. 2014. Detection of temporal trends in atmospheric deposition of inorganic nitrogen and sulphate to forests in Europe. *Atmospheric Environment* 95:363–374.
- Wallenstein, M. D., S. McNulty, I. J. Fernandez, J. Boggs, and W. H. Schlesinger. 2006. Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. *Forest Ecology and Management* 222:459–468.
- Weber, W. A. 1976. Rocky mountain flora. University of Colorado Press, Boulder, Colorado, USA.
- Williams, M. W., and K. A. Tonnessen. 2000. Critical loads for inorganic nitrogen deposition in the Colorado Front Range, USA. *Ecological Applications* 10:1648–1665.
- Wright, R. F., C. Alewell, J. Cullen, C. D. Evans, A. Marchetto, F. Moldan, A. Prechtel, and M. Rogora. 2001. Trends in nitrogen deposition and leaching in acid-sensitive streams in Europe. *Hydrology and Earth System Sciences* 5:299–310.

DATA AVAILABILITY

Data available from the LTER Network Data Portal: <https://doi.org/10.6073/pasta/1a11615f793bc5ca8b886aaa360ec615>.