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William M. Owens
University of Colorado Boulder, william.m.owens@colorado.edu

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Is Aluminum Toxicity a Mechanism of Change in Alpine Plant Communities in Response to Nitrogen Deposition?

By
William Owens
Ecology & Evolutionary Biology, University of Colorado at Boulder

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Thesis Advisor:
William D. Bowman, Ecology & Evolutionary Biology

Defense Committee:
William D. Bowman, Ecology & Evolutionary Biology
Barbara Demmig-Adams, Ecology & Evolutionary Biology
Abby Hickcox, Geography
Abstract

Deposition of nitrogen in many areas of the world is increasing with consequences for changes in plant community and ecosystem composition and function. Mechanisms underlying these changes include nitrogen fertilization, loss of base cations (positively charged ions that regulate changes in soil pH), soil acidification, and toxicity of metal ions like aluminum. I investigated aluminum toxicity as a possible mechanism of community change in alpine ecosystems in the Front Range of Colorado. I conducted a controlled greenhouse experiment using two dominant sedge species, *Kobresia myosuroides* and *Carex rupestris*. Presence of soluble aluminum in the soil did not affect growth of either species at any level of addition. These results are unique in that other aluminum manipulation studies have shown growth of plants (especially of roots) to be inhibited at lower levels, indicating these plants are resistant to aluminum toxicity. Other mechanisms that might have influenced the observed change of cover in field plots include competition for phosphorus and water, and loss of nutrient cations from the soil. Understanding belowground mechanisms of community shifts is important to predict future changes in areas with increasing nitrogen deposition.

1. Introduction

Levels of nitrogen deposition (NH$_4^+$, NH$_3$, NO$_x$, N$_2$O, NO$_3^-$) are increasing in some areas of the world (Galloway *et al.* 2008) and deposition is known to alter ecosystem composition and function (Fenn *et al.* 2003; Bobbink *et al.* 2010). Sources of this atmospheric pollutant include agricultural fertilizers and gaseous
emissions from the burning of fossil fuels (Galloway et al. 2008). Nitrogen deposition can alter ecosystems by increasing primary productivity (Thomas et al. 2010), decreasing plant diversity (Bobbink et al. 2010), and changing soil chemistry by removing base cations and acidifying the soil (Driscoll et al. 2001). While it is hypothesized that plant communities in ecosystems with high levels of nitrogen deposition shift to favor nitrophilic or acid-tolerant species (Stevens et al. 2010), there are few studies experimentally demonstrating primary mechanisms that mediate plant species and plant community responses to nitrogen deposition.

In alpine ecosystems on the Front Range of Colorado, nitrogen deposition does not induce a strong fertilization effect, where plants respond to increasing nitrogen availability by increasing biomass (Bowman et al. 2006, 2012), as is characteristic in other ecosystems (Stevens et al. 2010). This result suggests that competitive exclusion of some species by others as a result of shading may not be responsible for observed shifts in species composition, which have been observed under both ambient and experimental conditions of nitrogen addition (Bowman et al. 2006). Since the direct nitrogen fertilization effect does not appear to explain the observed trends of changing species abundances in alpine ecosystems, it is likely that other belowground factors are operating. Understanding the mechanisms of community change in alpine ecosystems is important for predicting how these ecosystems will change under predicted regimes of elevated nitrogen deposition (Fenn et al. 2003).

There are several indirect mechanisms by which elevated nitrogen levels can alter the soil environment and influence plant health. Leaching of nitrate (NO₃⁻)
from soils results in the loss of base cations (positively charged ions) because NO$_3^-$ bonds to cations in order to maintain electroneutrality, and these cations consequently are washed through the surface soil. Some of these base cations (e.g. Ca$^{2+}$, Mg$^{2+}$) are plant nutrients. Loss of these nutrients, therefore, could confer an advantage to plants better able to tolerate low-nutrient conditions or plants better able to take up or concentrate these cations.

Another mechanism of nitrogen altering soil chemistry is acidification and enhanced aluminum (Al) solubility. Acid buffering capacity in most soils is regulated by the amount base cations present. Without base cations, soil is unable to buffer the acidity of additional hydrogen ions, leading to acidification of the soil (Driscoll et al. 2001). As soil pH decreases, Al weathering increases, causing Al to become more soluble and enter the soil solution (Chadwick & Chorover 2001). The major effect of Al toxicity for plants is a decrease in, or cessation of, root growth. Al is thought to disrupt the functioning of the cytoskeleton of cells, and may change cellular levels of Ca$^{2+}$, among other possible mechanisms (Kochian et al. 2004). Previous nitrogen-addition experiments in plots in dry meadow alpine plant communities showed that acidification of the soil and increases in aluminum occur at a threshold of 28 kg N ha$^{-1}$ yr$^{-1}$, added as NH$_4$NO$_3$ in solution (Lieb et al. 2011). This is within the range of ambient nitrogen deposition predicted for this area by 2050 (Dentener et al. 2006). Differential tolerance of Al among plant species has not yet been explored as a potential mechanism underlying community change in alpine ecosystems, thus warranting my study.
In experimental plots in the alpine, the dominant sedge *Kobresia myosuroides* (referred to as *Kobresia* hereafter) has decreased in abundance and cover, and the sub-dominant sedge *Carex rupestris* (referred to as *Carex* hereafter) has increased in abundance and cover in response to nitrogen additions (Bowman *et al.* 2006). I examined whether differences in these species’ susceptibility to Al toxicity may be an underlying mechanism explaining the differential response to N deposition. I predicted that *Kobresia* would be less tolerant of increased Al availability compared to *Carex*, and would exhibit a greater inhibition of growth. To test this hypothesis, I set up a controlled greenhouse experiment with varying levels of Al additions and examined growth of the two sedge species.

2. Materials and Methods

2.1. Aluminum manipulation

In order to determine if increasing nitrogen addition might be contributing to changing sedge abundance via the pathway of Al toxicity, I assessed the direct effects of Al toxicity on the two focal species, *Kobresia* and *Carex* by comparing their performance under different levels of Al addition in a greenhouse experiment.

Live plants of both sedge species were collected from a dry meadow community on Niwot Ridge, northwest of the Mountain Research Station (MRS), CO. Blocks of alpine sod containing several sedge individuals were taken to the University of Colorado’s East Campus Greenhouse. Mature sedges were used instead of germinating seeds because these species grow too slowly to conduct the experiment on mature sedges grown from seed. Fifty plants of each species were
grown in native tundra soil collected on Niwot Ridge in 164 mL pots for four weeks. Sterile sand was placed at the bottom of each pot for drainage. During the experiment, day length and light levels were augmented with a High Intensity Discharge lamp to simulate natural changes in light availability during the growing season and to promote growth. Prior to initiating the Al treatments, plants received 20 mL water per pot every other day. Plants acclimated to greenhouse conditions for four weeks before Al treatments began.

Before starting the Al additions, the leaves of each plant were clipped to a length of 2.5 cm for consistency across species and treatments, so that each plant had roughly the same biomass at the start of the experiment. In addition, mass and length of the longest root were measured for each plant. The Al treatment solutions were added in the form of aluminum chloride hexahydrate (AlClO$_3$ $\cdot$ 6H$_2$O) at levels of 0, 100, 500, and 1000 μmol Al$^{3+}$ L$^{-1}$. These levels were selected based on previous experimental treatments examining Al toxicity in plants (Steiner et al. 2012; Batista et al. 2013). Twelve replicates per Al level and the control (water only) were maintained for each sedge species. Treatments were applied every four days, with all treatments receiving the same volume of treatment solution at the same intervals for 12 weeks. The experiment ended after 12 weeks to simulate the short alpine growing season. Soil pH was measured at the conclusion of the experiment using a Beckman 340 pH meter on 2:1 pastes of distilled water: fresh soil.

After 12 weeks, the longest leaf length was measured for each plant. Plants were harvested, gently washed, roots and shoots separated and placed in a drying oven at 60 °C for 48 hours. Root and shoot dry masses were then recorded.
2.2. Analysis

To evaluate the degree to which Al availability may influence plant growth, we ran linear regressions using aluminum level as a predictor for growth. We conducted linear regressions on leaf length, root mass, aboveground tissue mass, and root:shoot mass for Kobresia and Carex separately. All analyses were conducted in R (R Core Team 2012). We also conducted a Pearson’s correlation test to determine whether Al level induced the expected decrease in pH.

3. Results

Average growth of shoots over the course of the experiment was 6.3 cm for Carex and 5 cm in Kobresia. There were no significant effects of the Al treatments on any of the growth metrics for either species. Al had no effect on aboveground growth for either species. Shoot growth, estimated as the final shoot length (Kobresia: $R^2=0.015$, $P=0.375$; Carex: $R^2=0.033$, $P=0.218$), and root and shoot dry mass did not differ among the treatments for either species (Figures 1 and 2; Kobresia shoot dry mass: $R^2=0.003$, $P=0.687$; Carex shoot dry mass: $R^2=0.006$, $P=0.593$; Kobresia root dry mass: $R^2=0.015$, $P=0.388$; Carex root dry mass: $R^2=0.026$, $P=0.276$). Finally, root:shoot ratio was also unaffected by Al treatment level for each species (Figure 3; Kobresia: $R^2=<0.01$, $P=0.947$; Carex: $R^2=0.01$, $P=0.502$).
Figure 1: Aboveground dry mass for plants treated with 0, 100, 500, and 1000 μmol Al$^{3+}$ L$^{-1}$. Treatment level did not significantly impact shoot mass (Kobresia: $R^2=0.003$, $P=0.687$; Carex: $R^2=0.006$, $P=0.593$). Values are averages and bars are 2 standard errors of the mean.

Figure 2: Belowground dry mass for plants treated with 0, 100, 500, and 1000 μmol Al$^{3+}$ L$^{-1}$. Treatment level did not significantly impact root mass (Kobresia: $R^2=0.015$, $P=0.388$; Carex: $R^2=0.026$, $P=0.276$). Values are averages and bars are 2 standard errors of the mean.
Pre-treatment soil pH averaged 5.41 (standard error = 0.033, n=102). Soil pH decreased with increasing Al concentrations in pots with *Kobresia* (Figure 4; \( r = -0.22, P < 0.01 \)).
4. Discussion

Previous studies in experimental plots at the site where the sedges came from have shown a decrease in soil pH and increases in extractable Al with increasing nitrogen addition (Lieb et al. 2011). Studies in European grasslands growing on acidic soil parent material have concluded that acid and/or Al tolerance underlies the loss of diversity that accompanies increasing N deposition (Stevens et al. 2010). I predicted that higher levels of Al may be responsible for observed decreases in Kobresia and increases in Carex in experimental plots. Plant species less able to tolerate increases in Al should exhibit a decrease in cover in natural systems, while the converse would be true for species more tolerant of increases in Al (Kochian et al. 2004). However, not only was there no difference in growth between the two alpine sedges, neither species exhibited any sign of Al toxicity even at the highest levels of Al addition. The most common visible symptoms of Al toxicity established for other species are stunted and discolored roots, especially on lateral roots, as well as stunted and discolored leaves (Rout et al. 2001; Barceló & Poschenrieder 2002). We did not see any of these symptoms in the study sedges. Thus, Al toxicity does not appear to be a mechanism causing community change in alpine plants.

These results indicate that Kobresia and Carex from Niwot Ridge are remarkably tolerant to soluble soil Al. Al addition experiments on crop plants have shown significant decreases in growth at Al levels far less than the levels in our experiment (Tang, Nuruzzaman & Rengel 2003; Guo et al. 2004; Steiner et al. 2012; Batista et al. 2013). In a study on maize, for example, root growth was stunted at
levels as low as 75 μmol Al$^{3+}$ L$^{-1}$ (Batista et al. 2013). It is worth noting that both sedge species in our study are native to sites with acidic soils, and may thus experience high Al concentrations and a resulting greater selective pressure for Al tolerance. Their tolerance to Al may partially explain their dominance in this system. Work in the past several decades has characterized the alpine ecosystem as fragile (Zwinger and Willard 1996; Beniston 2003), but the present study and other studies (e.g. Spasojevic et al. 2014) indicate that the latter ecosystem may be quite resistant to at least some environmental stresses.

Mechanisms other than Al toxicity appear to underlie the observed changes in alpine plant community composition. Previous work (Lieb et al. 2011) in this system has shown that nitrogen fertilization causes loss of base cations, and different requirements for nutrient base cations among species may confer an advantage to species with lower requirements. However, the probable loss of base cations from the soils in our pot study did not appear to influence the growth of either species. Changes in plant-microbial interactions, such as changes in mycorrhizal infection or increases in pathogenic soil bacteria, have also been implicated as contributing to declines in species abundances (van Diepen et al. 2013, Ramirez et al. 2010). No significant changes in mycorrhizal infection or bacterial community composition were noted in the long-term nitrogen addition field plots (Potter et al. in prep). The available evidence supports competition as the most likely reason for the observed changes in the sedge species abundances. Significant negative correlations between the abundances of the two species have
been observed over the past six years, suggesting that competition may be occurring between the two species for water or phosphorus (Potter et al. in prep).

In experimental plots in the alpine, changes in composition of the dominant sedges *Kobresia* and *Carex* in response to nitrogen deposition have been observed, and my study indicates that aluminum toxicity is not a mechanism responsible for that shift. These sedges are quite tolerant to soluble soil Al, perhaps to a greater degree than would be expected based on other studies of plant Al toxicity. Other belowground factors should be more fully explored to explain shifts in community composition in this ecosystem.

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