EFFECTS OF NITROGEN ADDITION ON FINE ROOTS IN AN OAK FOREST

BRUCE MACHONA
Wiley College Marshall, TX USA 75670

MENTOR SCIENTIST: DR. GARY M. LOVETT
Institute of Ecosystem Studies, Millbrook, NY 12545 USA

Abstract. Burning of fossil fuels causes emission of nitrogen oxides, which can be deposited to ecosystems downwind of the emission sources. Excess nitrogen deposition to forests can have several effects; among them are changes in carbon allocation by the trees and increased leaching of nitrate through the ecosystem, which can cause depletion of base cations such as calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na). The depletion of base cations (BC) may cause acidification of the soil and increase aluminum (Al) concentration and mobility in the soil. Aluminum is antagonistic to BC in that it competes with and inhibits BC uptake by tree roots. Experimental addition of nitrogen to an upland oak-hickory forest near Millbrook, New York has resulted in increased tree mortality, but the cause of the tree death is not known. I investigated two hypotheses concerning how increased nitrogen supply could affect tree roots: (1) increases in nitrogen might cause trees to produce fewer fine roots and that could cause water stress; and (2) nitrogen can cause soil acidification, which can cause Al toxicity. Root samples were collected in six pairs of plots, with each pair consisting of a fertilized plot and an unfertilized control plot. Calcium and Al concentrations were measured on the fine (<1mm) roots and on samples of foliage taken from these plots in 1996 and 2002. I found that Ca concentration and Ca/Al ratio in roots were significantly lower in fertilized plots compared to unfertilized controls, but there was no significant difference in Al concentration. There were also no significant differences in biomass of live or dead roots. For foliage, there was no significant difference in Ca/Al ratio between fertilized and unfertilized plots in samples from 1996 (prior to the start of the fertilization) but the ratio was significantly lower in fertilized plots in 2002 (after 6 y of fertilization). Concentrations of Ca and Al in foliage showed no significant differences between fertilized and unfertilized plots either in 1996 or 2002. Thus, my study did not support the hypothesis that increased N caused changes in fine root biomass, but it did support the hypothesis that increased N led to potential Al toxicity, as evidenced by low Ca: Al ratios in roots and foliage, and perhaps to low Ca supply, as evidenced by the lower Ca concentration in roots. Although there is need to continue the investigations on the effect of nitrogen saturation on oak forests, these data suggest that excess nitrogen addition can lead to Al toxicity in roots, which can be detrimental to base cation uptake in trees.

INTRODUCTION

The atmospheric deposition of sulfur and nitrogen oxides, which are air pollutants derived primarily from fossil fuel combustion, can cause acidification of soils and surface waters. Although sulfur emissions have been declining in the eastern U.S. due to the Clean Air Act and its amendments, nitrogen oxide emissions have not declined in most areas (Driscoll et al 2001). The effects of nitrogen deposition on forest ecosystems are complex because nitrogen is both an important nutrient and a potential toxin for plants. Oversupply of nitrogen alter the normal patterns of nitrogen cycling in a process called "nitrogen saturation", which can produce leaching of excess nitrogen into groundwater, and potentially harm the surrounding trees (Aber et al 1998, Lovett 2004). Most studies have shown that nitrogen saturation results in NO_3^- and Al mobility in soils, causing soil and stream acidification. The acidic deposition changes the chemical composition of the soils by depleting the content of the available plant nutrient (i.e. Ca, Mg, K,) by increasing the mobility of Al and by increasing the sulfur and nitrogen content (Driscoll et al., 2001).

To investigate the effects of excess nitrogen deposition on forests, researchers at the Institute of Ecosystem Studies began a nitrogen fertilization experiment in 1996. The study is being done in mixed oak-hickory plots in an upland forest near Millbrook in the Hudson Valley of New York. Fertilization has dramatically increased the nitrogen leaching from these plots. Prior to fertilization, plots had low levels of ammonium (NH_4^+) and nitrate (NO_3^-) that were usually below detection. Measurable quantities of NH_4^+ and especially NO_3^- began to appear in soil solution in the fertilized plots soon after the fertilization began (Lovett 2004). Some trees in the fertilized plots are dying but the cause of death is unknown. High mortality began after two dry summers in a row (Lovett and Hart, pers. comm.).

While much research has been done on the effects of nitrogen fertilization on soils and foliage, fewer studies have been done on roots. Acidification of soils can alter fine root chemistry, which can influence the absorption of water and nutrients. Roots can actively exclude Al absorption by the plant, and the exclusion occurs between the root apoplast and symplast. Aluminum is believed to be toxic to plants by being antagonistic to base cations (Ca, Mg, K, and Na) in that it competes with and inhibits base cation uptake by roots.

The main goal of this study was to determine the effects of nitrogen fertilization on the fine root biomass and chemistry in these oak-hickory plots. In addition, I investigated the effects of fertilization on Ca and Al concentrations in oak foliage. . I hypothesized that (1) increases in nitrogen may cause trees to produce fewer fine roots and that could cause water stress; and (2) excess nitrogen can cause soil acidification, which can cause Al toxicity.

MATERIALS AND METHODS

Study Area and Experimental Design

The study was done in the upland forests of the Institute of Ecosystem Studies property near Millbrook NY. These forests are dominated by red oak (*Quercus rubra L*), chestnut oak (*Quercus prinus*) and various species of hickory with occasional individuals of red maple (*Acer rubrum*), sugar maple (*Acer saccharum*) and white pine (*Pinus strobus*) The bedrock is primarily slates and shales and the soils are thin, acidic, well drained silt loams (Glitzenstein et al 1990). Mean annual temperature at this site is 9.4 ^oC and mean annual precipitation is 1020 mm.

The fertilization experiment includes six pairs of plots consisting of six nitrogen fertilized and six unfertilized plots. Each plot is 20 m in diameter and the two plots in a pair are about five meters apart with fertilized downslope of the unfertilized plots. The plots have been fertilized with granular NH_4NO_3 since November 1996. The fertilizer has been applied in four equal doses per year for an annual application rate of 100 kg N ha^{-1} y^{-1} from 1996 through 1999 and 50 kg N ha^{-1} y^{-1} from 2000 through the present

Field Methods

Roots

Four-soil core samples, each 6. 5-cm diameter, were taken randomly from each plot in July 2004. The cores were 10cm deep, but only the upper 3cm of the soil, which represented primarily the organic horizons, were used in this study. The samples were carefully placed in a sealed plastic bag to be transported in a few hours to the lab and kept cool.

Foliage

The archived foliage samples from 1996 and 2002 were used to examine the Ca/Al ratio prior to and after fertilization. The 1996 samples were collected prior to fertilization of the plots and 2002 samples were collected after six years of fertilization. Both sets of samples were collected in mid-summer by shooting green foliage from dominant trees in the plot with a shotgun. The same trees were sampled in 1996 and 2000. The foliar samples were dried and ground and stored in paper envelopes.

Lab Methods

The roots were removed from the soils intact by repeatedly sieving the soils with a 2mm mesh. Sample was removed from the plastic bag in small amounts to keep the roots from drying out during the sorting. Roots dry out quickly during sorting and this makes it more difficult to determine live from dead roots. Tweezers were used to remove the soil particles from the roots and roots were soaked in the de-ionized water (DI) for 5 minutes to remove soil material attached to the roots. A magnifying glass with a light was used to help pick tiny root samples from the sieved soil. A 1 mm diameter wide wire was used as a guide to help distinguish fine roots (< 1mm) in diameter. The dead roots were determined by texture: they are either brittle or snap easily (like a dead twig) or spongy and partially decomposed. Dead roots also float; live roots generally sink. However, this process of separating the dead roots from the live was not 100% satisfactory, as even the live tiny fine roots can float. Sorted roots were placed into pre-weighed vials, weighed and stored in the refrigerator until ready for drying. The roots were oven dried at $70^{\mathbf{0}}$C for 48 h then removed from oven and weighed to .0001g.

Both the root and the archived foliage samples were ground to powder using a KLECO Ball Mill. Subsamples of 0.5g were placed into an acid washed crucible for ashing, then samples were ashed at 475^0 for four hours. The foliar samples were ashed at 500^0 for twelve hours. The extraction of Ca and Al from the samples was done using concentrated HNO_3 . Eight ml of 6N HNO_3 were poured into a crucible with 0.5g of ashed sample for digestion. The digestion was done using a hot plate stove with crucibles placed a pan with a layer of sand to keep the crucibles up straight. The crucibles were removed from the hot plate as soon as they started simmering, filtered through Whatman 41 filter paper into acid-washed 50ml flasks and then poured into acid-washed plastic bottles. Samples were stored in a refrigerator prior to analysis.

In both the root and foliage samples, the Ca and Al concentrations were analyzed using an inductively coupled plasma emission spectrometer (ICP). Each sample batch was concurrently run with the pine needle reference (No.1575) and apple leaves reference number (No. 1515) available from National Institute of Standards and Technology (NIST). The mean total Ca recovery rates for nutrient analysis of NIST standard pine needles and apple leaves were 88% and 86% respectively. The Al recovery rates for pine needles and apple leaves were 68% and 80% respectively. The reported concentrations in the root and foliar samples were corrected for incomplete recovery using the percent recovery in the apple leaves. We did not use HCl in the extraction of Al from the plant tissue, which may be the reason for the low recovery of Al in these analyses.

Statistical analysis

Effects of the fertilization treatment on root biomass and chemistry were evaluated using analysis of variance, using fertilization and site (i.e. plot pair) as main effects and also evaluating the interaction term. For the foliar chemistry, for which we had only one sample per plot, fertilization effects were evaluated using a paired t-test.

RESULTS

There was no significant effect of fertilization on the biomass of live (p=0.43) or dead (p=0.13) fine roots or the live/dead ratio (p=0.28) (Figure 1). Likewise, there was no significant interaction between fertilization and site. As expected, the Ca concentration in roots was significantly lower in fertilized plots compared to unfertilized plots (Figure 2A). However, there was no significant difference in root Al concentration between fertilized and unfertilized plots (Figure 2B). As expected, the Ca/Al ratio in roots was significantly lower in fertilized plots compared to unfertilized controls (Figure 2C). The fine root Ca/Al ratio averaged 0.65 (±0.27 s.d.) in unfertilized plots and 0.39 (±0.15) in fertilized plots.

For foliage, there was no significant difference in Ca/Al ratio in samples from 1996, but the ratio was significantly lower in fertilized plots in 2002 (after 6 years of fertilization) (Figure 3). The Ca: Al ratio declined over this time period in both fertilized and unfertilized plots, but the variance among plots was less in 2002, leading to the significant difference.

DISCUSSION

We found that this fertilization treatment produced differences in chemistry, but not biomass, of fine roots. Thus the results do not support hypothesis 1 (that fertilization will reduce fine-root biomass) but they do support hypothesis 2 (that fertilization will reduce fine root Ca/Al ratios).

In hypothesis 1 I expected that increases in N may cause trees to produce fewer fine roots and that could cause water stress. Roots rapidly proliferate in zones where limiting nutrients are available (Pregitzer et al. 2001). In a series of fertilization and N exclusion studies in Europe, fine root biomass increased significantly following nitrogen exclusion from a site with high nitrogen deposition (Boxman et al. 1998, Gundersen et al.1998). However, there were no significant differences in root biomass between fertilized and control plots in sites where N fertilizer was added. In our study, the results might have been influenced by the difficulty in distinguishing dead from live roots, especially as the roots began to dry out during the sorting process. Because fine roots <1mm in diameter are the primary organs involved in nutrient absorption, it is important to investigate their biomass. It may be useful to study fine roots using other methodologies such as minirhizotrons, where images from belowground are videotaped and analyzed.

The lower Ca concentration in the roots of the fertilized plots suggest that base cations (BC) are being mobilized and lost from the plots due to N saturation in the soil. Chronic nitrogen additions could increase net nitrification, or induce it where previously absent, and nitrate leaching losses would increase (Aber et al 1998). Leaching of nitrate through the ecosystem can deplete cations such as Ca and increase Al concentration. The increases in Al concentration will hinder and deter BC uptake by roots resulting in deficiency of nutrients (Cronan and Grigal 1996). However, we did not find higher Al concentration in roots from fertilized plots compared to unfertilized plots. Many investigators believe that the Ca/Al ratio in soil solution or in plant tissue is a good indicator of Al stress on plants (Cronan and Grigal 1995). Sixty percent of the studies reviewed by Cronan and Grigal (1995) showed adverse effects on plants when root Ca/Al ratios were less than 0.2. However, in this study the Ca/Al molar ratios in both fertilized and control plots were above 0.2. The Ca/Al molar ratios in the fertilized and unfertilized plots were 0.39 and 0.65 respectively (Figure 2C), values which are nonetheless low compared to non-acidified sites in the literature (Joslin et al 1988, Cronan and Grigal 1995, Joslin and Wolfe 1989).

Despite the fact that the Ca/Al ratios are >0.2, our observations indicate that fertilized plots have greater tree mortality than unfertilized plots. One possible explanation for the death of trees in fertilized plots is the aftereffects of the droughts experienced 1999, 2001 and 2002. The sudden changes in environmental condition could have stressed the trees in fertilized plots due to lack of nutrients as a result of Al toxicity. Also, the Ca/Al ratio could be lower in a drought year compared to a wet year like 2004. The results suggest that a root Ca/Al

ratio of <0.2 may be insufficient as an indicator of acidification stress on trees because the critical ratio may vary substantially from one place to another and among different species. However, the Ca/Al ratio in roots may still be a useful indicator for identification of approximate thresholds beyond which the risk of forest damage from Al stress and nutrient imbalances increases (Cronan and Grigal 1995).

In the foliage samples, there was no significant difference in chemistry between fertilized and unfertilized plots in 1996 because the samples were taken before the fertilization began. The high variability in Ca/Al ratio for 1996 is likely due to natural variation in soil acidity. However, the 2002 results, after 6 years of fertilization, show significant difference in foliar Ca/Al ratio between fertilized and unfertilized plots. However, the Ca/Al ratio declined in both fertilized and unfertilized plots between 1996 and 2002, whereas I would have expected it to remain constant in the unfertilized plots. This suggests substantial interannual variability of Ca/Al ratios and indicates that a longer series of measurements may be necessary to determine reliable average values.

CONCLUSIONS

The results of this study suggest that there is no significant fertilization effect on the biomass of live or dead roots. Thus, my study did not support the hypothesis that increased N caused changes in fine root biomass. However, this study did support the hypothesis that increased N led to potential Al toxicity, as evidenced by the lower Ca/Al ratios in roots. Fertilization with N is one of the anthropogenic processes that can contribute to biotic stress in forest ecosystem, by depleting soil nutrients and promoting soil acidification. More research is needed to determine the effects of lower, chronic doses of N such as those presently occurring from atmospheric deposition, and to examine in more detail the factors controlling fine root biomass.

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APPENDIX

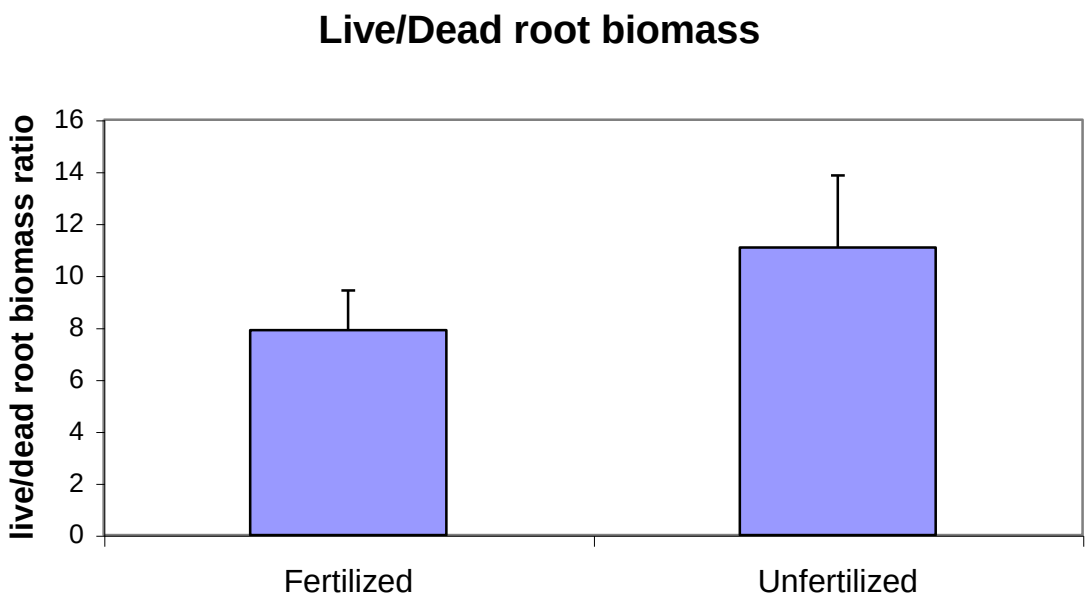

FIGURE 1. Ratio of live/dead fine root biomass in fertilized and unfertilized plots. Bar heights are means and error bars are standard errors. Difference in means is not statistically significant.

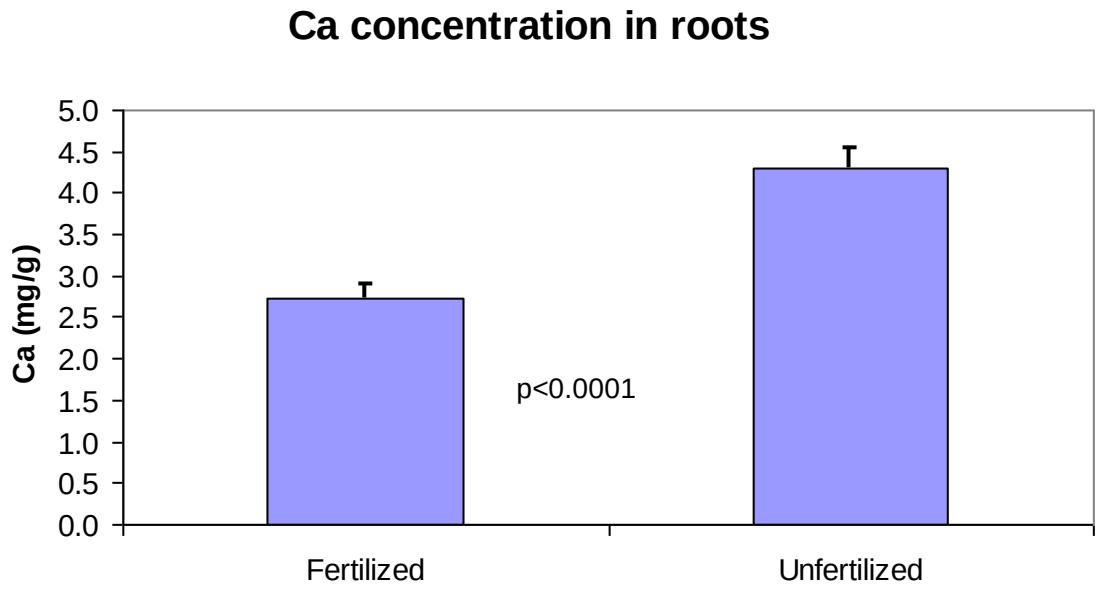

FIGURE 2A. Fine root Ca concentration in fertilized and unfertilized plots. Bar heights are means and error bars are standard errors.

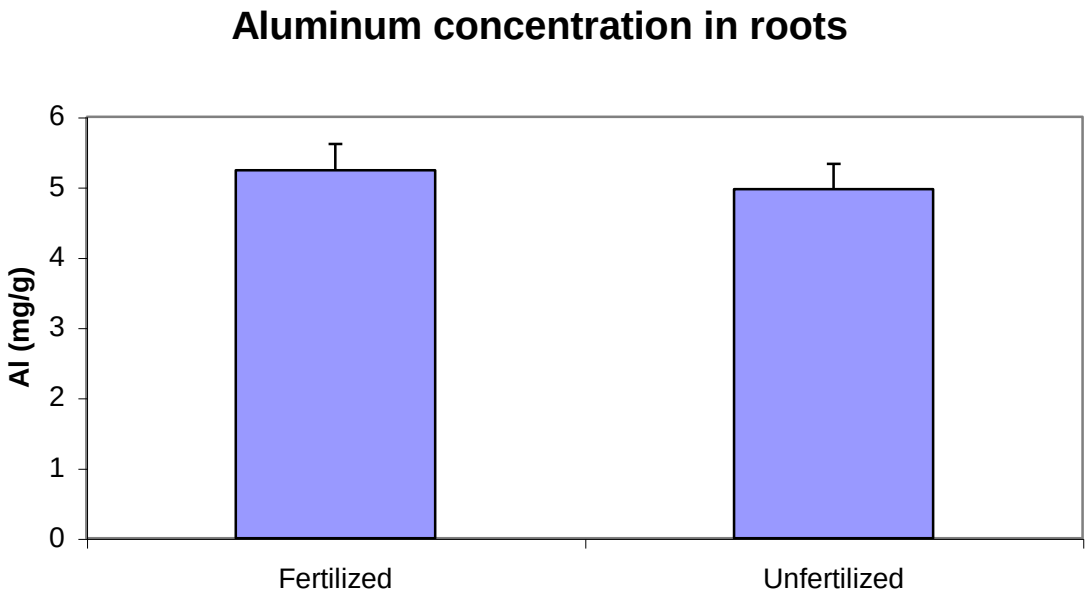

FIGURE 2B. Mean (±s.e.) Al concentration in fine roots in fertilized and unfertilized plots. Difference in means is not statistically significant.

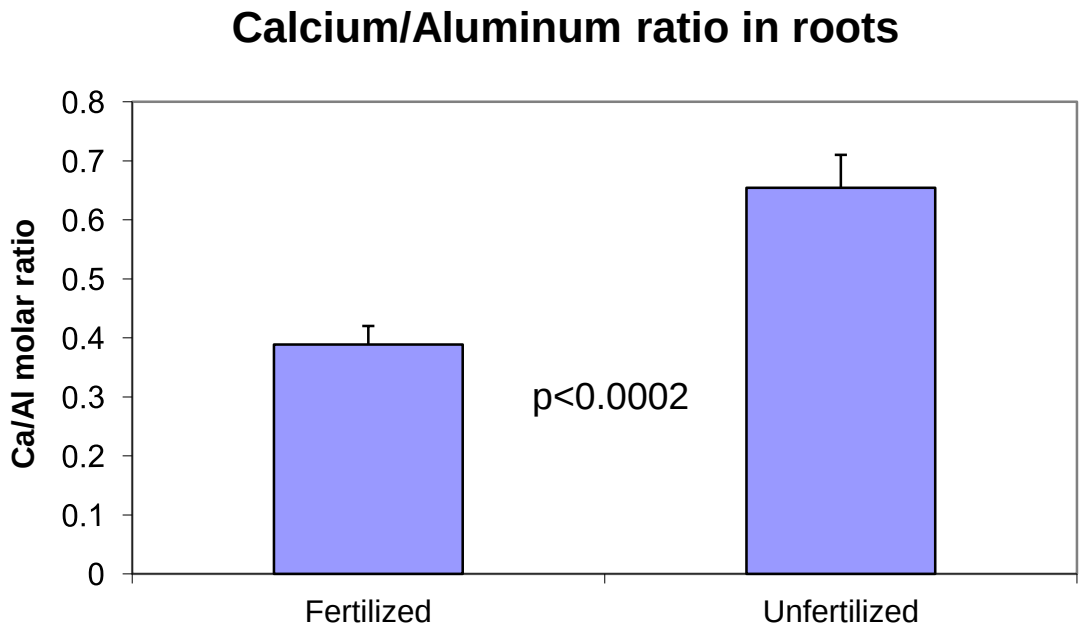

FIGURE 2C. Mean (±s.e.) Ca/Al ratio in fine roots in fertilized and unfertilized plots.

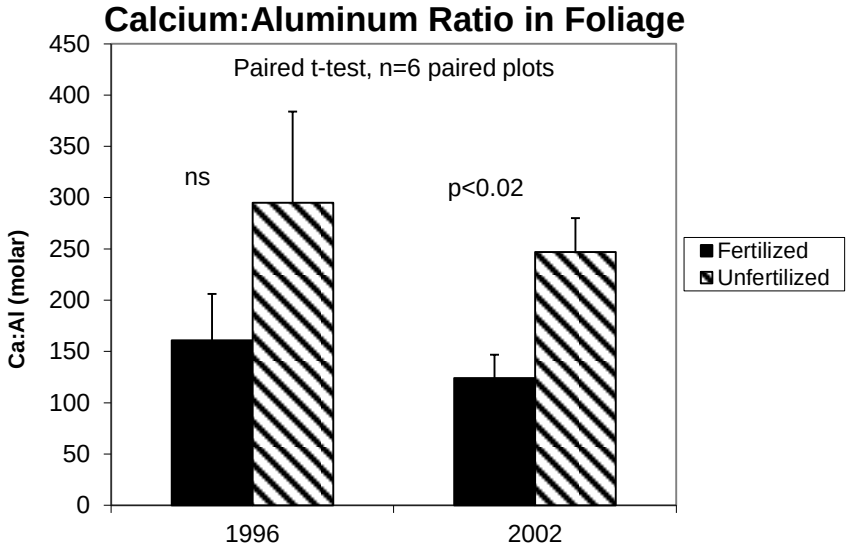

FIGURE 3. Mean (±s.e.) Ca/Al ratio in archived red oak foliage in fertilized and unfertilized plots for years 1996 and 2002.