

Research Article

Calorespirometry Measurement of Sugar Maple Seedling and Mature Tree Response to Calcium, Magnesium, and Nitrogen Additions in the Catskill Mountains, NY, USA

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Abstract

Sugar maple is an important component of northeastern North American forests that has been declining for several decades. Sugar maple decline may alter stand structure and long-term stability of its habitat. The decline may be related partly to the effects of acid deposition on soil nitrogen (N) and base cations such as calcium (Ca) and magnesium (Mg). Calorespirometric measurements of rates of plant metabolic activity have been suggested as a rapid measure of plant response to environmental conditions. Instantaneous rates of metabolic heat production (R_q), CO_2 production (RCO_2), and metabolic efficiency (R_q/RCO_2) were measured on leaf tissue of *Acer saccharum* Marshall (sugar maple) seedlings and mature trees, during two summers, in 12 forest plots in the Catskill Mountains, NY, in response to a 2-way factorial combinations of N and Ca+ Mg addition to forest soil. Contrary to our expectation, no beneficial effect of Ca+ Mg addition was detected in terms of increased metabolic rate of sugar maple seedlings or mature trees. N addition decreased the metabolic rates in mature trees, suggesting that N addition can decrease the long-term growth potential of mature sugar maple trees in our study site, which is reported to be N saturated due to continuous atmospheric N addition.

INTRODUCTION

This manuscript is published posthumously in memory of Dr. Bahram Momen (1955-2018), who directed this study. His service and dedication to his student's, colleagues and his profession made possible this and dozens of other manuscripts. He will be remembered as a valuable colleague and leader, a selfless person who never said "no" to a student who needed his help and a good friend.

Acer saccharum Marsh (sugar maple) an important ecological and economical tree species found throughout northeastern North America's forests has been declining for several decades [1-6]. Sugar maple decline can alter the structure and long-term stability of its habitat [7] and may be related to acidic deposition that can affect the availability of soil nutrients such as nitrogen (N), calcium (Ca), and magnesium (Mg). Detection of sugar maple decline requires a long-term monitoring of under- and over-story stand overall growth. However, such long-term, overall growth depends on the short-term alteration of plant eco-physiological processes such as carbon gain and metabolic

activities that are affected by changes in soil chemistry. N is an essential component of proteins involved in photosynthesis and respiratory metabolism, and is often a growth-limiting nutrient in forests. However this may no longer be the case in area such as the Catskill region of New York that have been receiving high atmospheric deposition and may be nitrogen saturated [8].

Depletion of soil calcium by acidic deposition has been reported for sugar maple stands [9,10]. Sugar maple has a high demand for Ca, which is required by a number of plant eco-physiological processes and its depletion can harm sugar maple health and regeneration [5,6,11,12]. Therefore, Ca addition may help stressed sugar maple stands by enhancing leaf area, fine twig growth, seed production, and root starch concentration [13-17].

Results of linking growth or decline of sugar maple to soil N concentrations have been inconclusive [4,7,18,19]. Moreover, there has not been any study of the effects of N, Ca, and other basic cations such as Magnesium (Mg) in a factorial setting in the field to quantify their additive or interactive effects. This is particularly important for both seedlings and mature sugar

maples considering the fact that seedling responses may not adequately reflect mature tree responses. Our main objective in this study was to quantify the synergistic or interactive effects of a factorial combination of N and Ca+ Mg additions on both sugar maple seedlings and mature trees. Quantification of the treatment effects on stand growth and health requires a long-term monitoring program, particularly for mature trees, and it would be desirable to assess possible treatment effects within a shorter time span.

Calorespirometry is a promising method of quantifying plant respiratory rate and substrate carbon conversion efficiency for early detection of the effects of stressful or favorable environmental conditions. However, plant eco-physiology literature has focused mostly on gas exchange measurements of net photosynthesis, CO₂ production, and or O₂ consumption rates. Under favorable conditions, plant respiratory heat rates can be used to indicate the sink activity and efficiency of converting photosynthate to structural components, and thus, realized growth and health condition [20,21]. Assessing metabolic rate by the usual measurement of the rate of CO₂ evolution in the dark (dark respiration) may not reflect the total metabolic rate accurately because some intermediates of respiratory carbon metabolism can be diverted into synthetic reactions and never appear as CO₂. Also, the energy use efficiency of different synthetic pathways using respiratory products varies [22-24]. Calorimetric measurements of metabolic heat production (*Rq*) can be a more accurate measure of total metabolism with an emphasis on oxidative reactions [25,26]. Measures of *Rq* per unit of CO₂ evolution or O₂ consumption can also be used to detect thermodynamic efficiency of metabolic reactions and quantify catabolic and anabolic components of respiration and their ratio to determine if a plant is experiencing favorable conditions and growing or if it is stressed and in decline. Under optimum environmental conditions, if the metabolic efficiency does not change, increased *Rq* should indicate increased growth potential. Positive correlations between different measures of growth have been reported previously [21,26-28].

The calorespirometry theory and methodology are thoroughly discussed elsewhere [29] and explained briefly in the Material and Methods section. We quantified instantaneous rates of respiratory metabolic heat production (*Rq*), CO₂ production (*RCO₂*), and metabolic efficiency (*Rq/ RCO₂*) in response to N and Ca +Mg addition to forest soil, during two summers, in 12 forest plots in the Catskill Mountains, NY. We hypothesized that Ca addition could increase both *Rq* and *RCO₂*, indicating sugar maple enhanced potential growth and release from stress caused by base cation depletion due to acidic deposition. We also hypothesized that N addition would not have any positive effect on the responses measured because the study site is reported to be N saturated.

MATERIALS AND METHODS

Study site

The study was conducted in the Neversink River drainage in the Catskills region in southern New York, USA. The Catskills region is an important ecological site providing drinking water for New York City. Catskills' soil is characterized mainly as

inceptisols that are susceptible to ion imbalances and pH changes due to acidic deposition. Parent material of this soil is 40% mudstone and siltstone and 60% sandstone and conglomerates [30] that provide a very low concentration of base cations to the soil. Inceptisols are sandy loams with an average of 60% sand, exhibiting well-drained porous characteristics that result in easy leaching of nutrients [31,32]. Additionally, the Catskill soils are acid-sensitive due to glaciation, which scoured exchangeable base-rich soils from ridge tops and slopes.

Experimental design and treatments

A randomized complete block design (RCBD) with three blocks each at three elevations, 670-680, 700-710, or 720-730 m was used. Within each block, four 50x50 m plots were assigned randomly to each of the four treatments consisting of: 1) reference (no additions), 2) annual additions of 25 kg ha⁻¹ of N for three years(+N), 3) a single addition of 2268 kg of dolomitic limestone in the fall (+Ca Mg) and 4) a single addition of 2268 kg of dolomitic limestone in fall plus annual additions of 25 kg ha⁻¹ of N for three years (+CaMgN). N was added as NH₄NO₃ for two years, and then in the form of urea thereafter when NH₄NO₃ became unavailable commercially. The addition of 25 kg N ha⁻¹ was approximately double the atmospheric deposition of N at the site. The dolomitic limestone was 17.7% Ca and 10.6% Mg, which resulted in additions of 1606 kg Ca and 962 kg Mg ha⁻¹. Both the dolomite and N were applied in pelletized form by hand. To ensure uniform distribution, each plot was divided into 5x5 m squares to receive a pre-measured dose.

Foliage sampling and measurements

Foliage was collected from mature trees and seedlings in the field during July mornings in 2006 and 2007. During each measuring period, foliage was harvested from three mature trees (as subsamples) from each replicate plot. Few seedlings were available, and thus, only one seedling per replicate plot was sampled. Foliage from mature trees was collected by shotgun sampling of branches from the periphery of upper canopy positions from dominant or co-dominant trees. The foliage was immediately put in air-tight plastic bags inside a cooler at 1C and transported to a refrigerator in the laboratory.

A multi-cell differential scanning calorimeter (MC-DSC, TA Instruments, New Castle, DE) was used in isothermal mode. This device has four independent slots with 1 mL sealed ampules that enables measurements on three samples at a time with the fourth serving as a reference. The measuring temperature was set between 20-25 C to mimic the average midday temperature in the field at the time of sampling. One set of measurements was made first with empty ampules to obtain an instrument baseline for each slot (in μ W) to subtract from foliage measurements. Approximately 150 mg (fresh weight) of leaf foliage with minimal visible injury, usually from the center, was cut with a sterile razor and put inside each measuring ampule with a small piece of wet paper tissue to minimize tissue evaporation. The ampules were sealed and placed in the calorimeter and allowed to equilibrate for about 20 minutes at the controlled temperature. Then, heat production rates were measured to reach constant values within about another 20 minutes. Next, the ampules were taken out, opened, and a small vial containing 50 μ L of 0.4 M NaOH was

inserted into each of the ampules. The ampules were resealed and placed inside the calorimeter again, and heat production rates measured until constant values were reached. The CO₂ produced by respiration inside the ampules reacts with the NaOH and produces 108.5 kJ/mole CO₂ in addition to the heat from metabolizing foliage [32]. Finally, the NaOH was removed from the ampules and another set of heat rate measurements made on the foliage alone to adjust for possible time effect on the foliage heat production rate. After the heat rate measurements, the foliage was oven dried at 70°C for three days to obtain constant dry mass. The completion of all subsamples and replicates of mature-tree and seedling foliage required 4 to 5 days of storage of leaves after harvesting. A preliminary experiment indicated no significant effect of storage time on our sugar maple foliage. Lack of storage time effect is also reported for ponderosa pine [33].

Statistical analysis was performed using The MIXED Procedure of the SAS System testing for main effects of N addition and CaMg addition and their interaction in a 2X2 factorial.

RESULTS AND DISCUSSION

Metabolic heat rate (*Rq*) and the rate of CO₂ production (*RCO₂*) were normalized per unit of dry mass, and *Rq/RCO₂* calculated as described by Hansen et al. [33]. We used many subsamples but the statistical analysis was performed based on three replicated forest plots for each treatment combination. These results are shown in Figures 1-3.

No interaction (*P* > 0.05) was detected between the effects of CaMg (liming) and N addition on any response measured for seedlings or mature trees. Therefore, the main-effect means of each level of either liming or N addition were averaged across the other factor. These are reported in Figures 1-3. No effect of liming or N addition was detected for seedlings on any metabolic response measured. In mature trees, N addition decreased rates of foliar metabolic heat production (Figure 1) and, CO₂ production (Figure 2), but had no significant effect on metabolic efficiency (*Rq/RCO₂*-Figure 3) (*P* < 0.5).

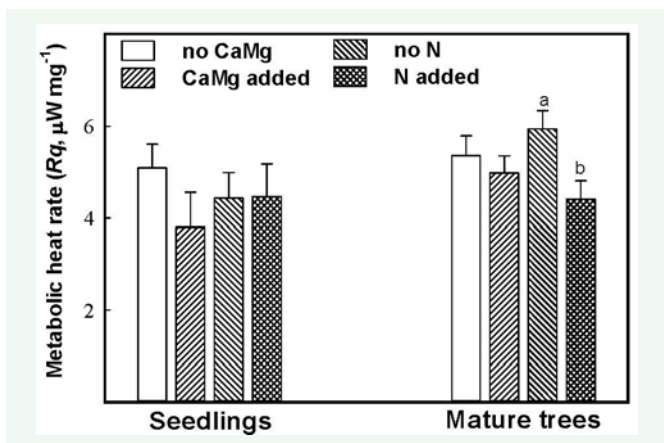


Figure 1 Main-effect means of addition of either N (with and without CaMg added combined, “no N” or “N added”) or addition of CaMg (with and without N added combined, “no CaMg” or “CaMg added”) on foliar metabolic heat production rate. Each bar is the mean with standard error (arrows) of at least three samples. Different letters on top of bars (SEs) indicate a statistically significant (*P* < 0.05) difference in means.

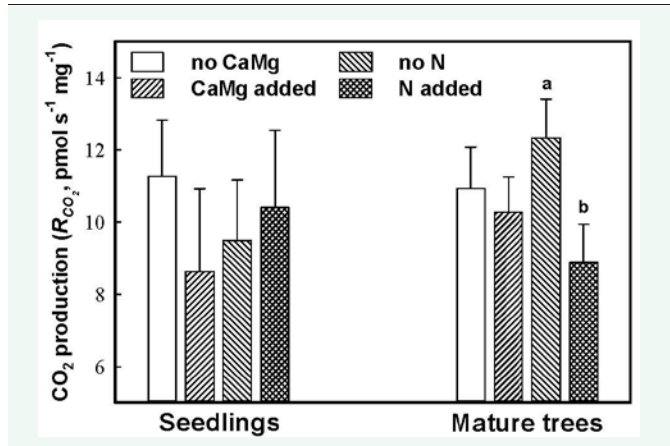


Figure 2 Main-effect means of addition of either N (with and without CaMg added combined, “no N” or “N added”) or addition of CaMg (with and without N added combined, “no CaMg” or “CaMg added”) on foliar CO₂ production rate. Each bar is the mean with standard error (arrows) of at least three samples. Different letters on top of bars (SEs) indicate a statistically significant (*P* < 0.05) difference in means.

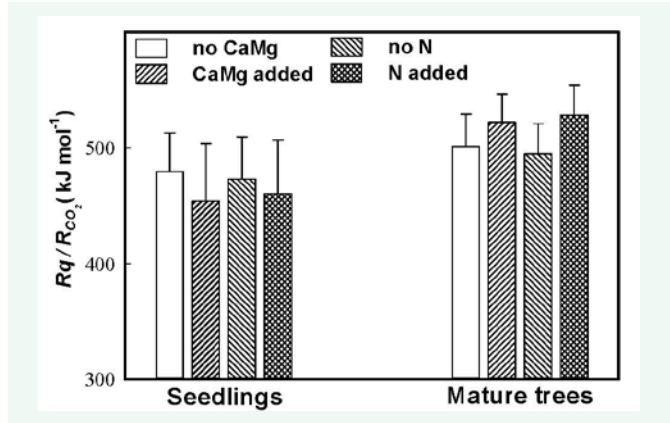


Figure 3 Main-effect means of addition of either N (with and without CaMg added combined, “no N” or “N added”) or addition of CaMg (with and without N added combined, “no CaMg” or “CaMg added”) on foliar metabolic heat produced per mol CO₂ released production rate. Each bar is the mean with standard error (arrows) of at least three samples.

No statistical comparison was made between seedling and mature responses as this was not our objective. However, the measured responses of seedlings and mature trees did not seem to differ. *Rq/RCO₂* seemed to be higher in mature trees than in seedlings, but both sets are within the limits of error of the 470 kJ/mole expected for non-growing tissue respiring sugar [34,35].

Contrary to our expectations and previous reports in the literature [36], calcium and magnesium addition did not induce a positive foliar metabolic response in seedlings or mature trees (Figure 1-3). This was unexpected especially for seedlings considering that liming increased Ca, Mg, and pH in the soil Oe horizon [37] where fine roots of seedlings are present. This indicates that liming may not benefit sugar maple seedlings’ establishment and survival. Mature trees have the bulk of their roots in a deeper horizon for which soil chemistry data before and after treatment application are not available, and therefore,

discussion of lack of liming effect on mature trees is not relevant. However, levels of foliar N were shown to increase in mature sugar maples in response to liming and this was accompanied by a reduction in C:N ratio in foliage [38].

In interpreting the results, we assume that the decreased metabolic rates in mature leaves adversely affected transport of photosynthate to stems and roots. Although N addition did not affect soil Oe, Oa/A, and B horizons [37], it adversely affected mature trees by decreasing their instantaneous rates of foliar metabolic heat production (Figure 1) and CO₂ production (Figure 2). These adverse N effects on mature trees are consistent with a decrease in apparent quantum efficiency of mature trees, but contrary to the result that N, when combined with Ca and Mg addition, increased photosynthetic capacity of mature trees [37]. However, the positive or negative effects of N, Ca, and Mg addition on the short-term, instantaneous eco-physiological responses did not result in changes in the basal area of mature sugar maple trees in our forest sites [37].

The effects of N and liming on sugar maple have been studied widely but no past study has focused on the metabolic responses measured in this study. Our results provide a small step towards understanding the complex issue of the adverse effect of atmospheric deposition and climate change on sugar maple mature tree die-back and lack of seedling establishment.

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