ABSTRACT

Title of Document: EFFECTS OF NITROGEN AND CALCIUM ON PHOTOSYNTHESIS AND METABOLIC ACTIVITY IN ACER SACCHARUM IN THE CATSKILL MOUNTAINS.

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Plant Sciences and Landscape Architecture

The Catskill Mountains in southern New York have received some of the most acidic rainfall in the country for the past 50 years. Acid deposition on these thin soils may deplete the concentration of calcium and other ions in the soil solution and mobilize other ions that can be harmful to sugar maple (*Acer saccharum*) rooting systems. The effects of fertilizers on the metabolism and photosynthesis rates of sugar maple are of great interest to both farmers and ecologists.

In this study, 12 plots in a 60-year-old sugar maple dominated forest were treated with no fertilizer, nitrogen, calcium, or nitrogen and calcium together. Photosynthesis was measured with a LiCor 6400. Metabolic heat rate was measured with a MC-DSC calorimeter. While some sampling periods showed significant responses to some treatments, the study as a whole suggests the addition of calcium and/or nitrogen had minimal effects on photosynthesis or metabolism.

EFFECTS OF NITROGEN AND CALCIUM ON PHOTOSYNTHESIS AND METABOLIC ACTIVITY IN ACER SACCHARUM IN THE CATSKILL MOUNTAINS

By

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Chapter 1: Introduction

The temperate broadleaf and mixed forests of northeastern North America stretches over 65 million hectares throughout the United States and Canada and surround some of the most populous metropolitan and industrial regions in the US. Sugar maple, *Acer saccharum*, is vital for lumber, syrup, and $2.5 million in fall tourism (US Department of the Interior, 1991). Since sugar maple has the unique combination of being not only the dominant species of mountainous Northeastern forests but also a keystone and indicator species in this region, sugar maple health is important to the understanding of this entire ecosystem (Driscoll et al., 2001).

After Tomlinson's finding (1990) that a number of sensitive species of trees were showing decline in Eastern US forests near industrial centers and major metropolitan areas, a considerable amount of recent attention has been turned towards the decline in the reproduction and photosynthetic productivity of sugar maple (Bernier and Brazeau 1988, Adams and Hutchinson 1992, Kolb and McCormick 1993, Ouimet 1995, Wilmot et al., 1995, Horsley et al., 2000, Wargo et al., 2002, Driscoll et al., 2001). Decline has been defined as a gradual general lack of vigor resulting from the interaction of biotic or abiotic stressors, often ending with the death of the tree (Manion and Lachance 1992). Sugar maple decline has been noted for the past century, but it was not until Giese conducted a systematic study of declining sugar maples in Wisconsin that thorough documentation of the symptoms of decline took place (1964). Spurred by Giese's study, Mader and Thompson (1969) outline the factors included in sugar maple decline in the Northeast, where the species was struggling even more than what Giese had observed in the Midwest. Since that time,

these signs of decline have been well documented and have included crown dieback, chlorotic foliage, seedling mortality, poor production of samaras, reductions in growth, and most dramatically, tree mortality (Fig. 1.1) (Manion and Lachance, 1992; Long et al., 1997). Decline has been documented throughout Eastern North America, including Massachusetts (Mader and Thompson 1969), Ontario (Henderson and Jones 1989), and Quebec (Bernier and Brazeau 1988, McLaughlin et al., 1987, Long et al., 1997, Watmough et al., 1999), but most dramatically in Pennsylvania and New York (Colb and McCormick 1993, Jones and Hendershot 1989, Horsley et al., 2000, Cote and Camire, 1995, Ouimet and Camire 1995, Wilmot et al., 1995).

Sugar maple decline has been attributed to many factors including herbivory by insects or vertebrates, and changes in weather patterns. Both exotic and native insect species are suggested to contribute to defoliation, a major biotic stressor in populations (Kolb and McCormick 1993, Wargo et al., 2002). For example, sugar maple borer (*Glycobius speciosus*), forest tent caterpillar (*Malacosoma disstria*), pear thrips (*Taeniothrips inconsequens*), and gypsy moth (*Lymantria dispar*) have defoliated at least 90% of sugar maple stands at least once since 1980 in the region (Stout et al., 1995, Rhoads and Pallardy 1993). Vertebrate browsing is also of concern, especially as deer and rabbit populations continue to explode in both suburban and rural areas. As suburban sprawl continues to encroach on wild areas and habitat fragmentation increases in Southern New York State, herbivores such as these continue to thrive in areas where predators and disease vectors are becoming increasingly rare (Didier and Porter 2003). Deer are of most concern among herbivores because of their preference for young sugar maple shoots during the

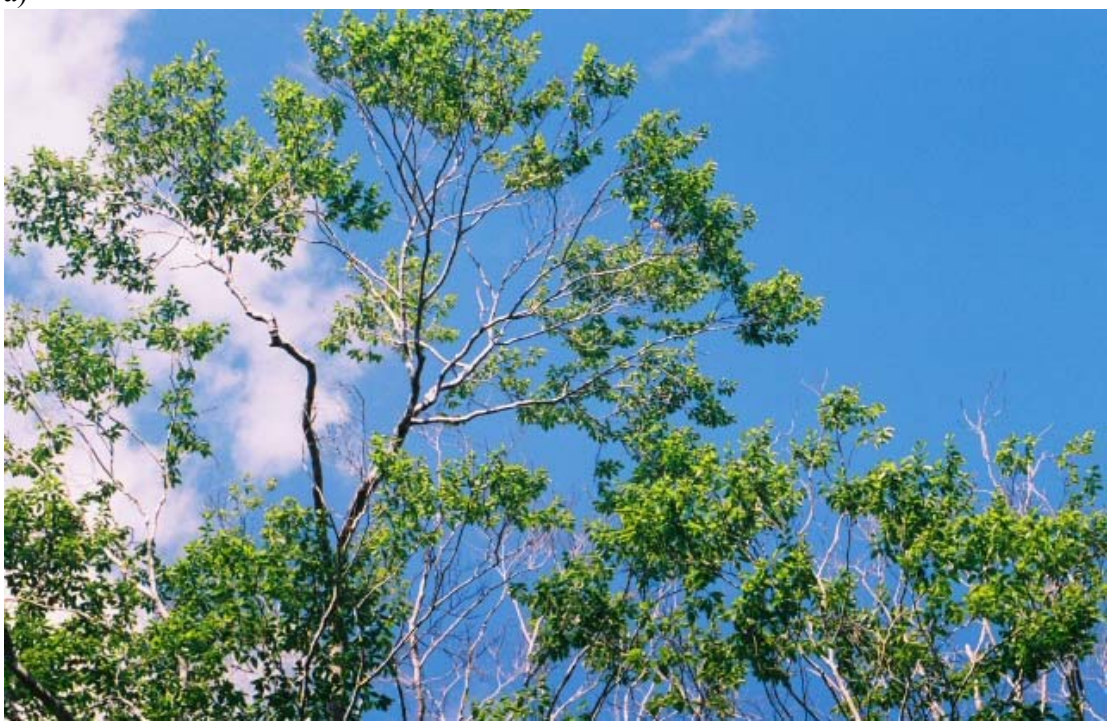
autumn and winter months and their ability to decimate an entire individual sapling at once (Didier and Porter 2003).

In addition to these biotic stresses, changes in climate and local weather patterns may also be contributing to sugar maple decline. The Catskills are experiencing increasingly higher than normal winter and summer temperature cycles and a mean increase of .6 degrees Celsius over the past 50 years (Burns et al., 2007). This had led to a decrease in total annual frost days which has been hypothesized to have a strong negative impact on sugar maple root systems (Tierney 2001). Snowpack usually insulates the soil and prevents upper horizons from freezing (Stadler et al., 1996; Shanley and Chalmers 1999). Without the snowpack, frost has been shown to damage fine roots and eventually cause crown dieback (Boutin and Robitaille 1995; Robitaille et al., 1995) (Fig. 1.1 b).

Drought also has a negative affect on sugar maple productivity, and the reductions in summer precipitation in the Catskills (Kolb and McCormick 1993, Allen et al., 1992) when transpiration is at is highest has also been shown to be a stressor (Allen et al., 1992, Kolb and McCormick 1993). The majority of sugar maple root mass is found in the upper 20 cm of soil, making it very susceptible to drought (Ni and Pillardy 1993). In addition to the direct physiological damage by drought, the lack of soil solution may have a secondary effect by reducing nutrient uptake if soluble nutrients such as nitrogen, calcium, sulfur and magnesium are not present in the aqueous rhizosphere (Barber 1995, Roth and Fahey 1998, Jackson et al., 2004).

Fig. 1.1. Commonly found signs of decline seen in sugar maple stands in the Catskill Mountains; fine twig death in midsummer (a), and browning foliage in a stands' canopy as a result of extreme insect herbivory (b).

a)



b)

An increase in soil temperature could also affect nutrient cycles. For example, Verburg has shown a significant increase in nitrogen mineralization in some studies (2005) and none in others (Groffman et al., 2001), although an increase in nitrate seems universal in these studies. Warmer winters could also contribute to reduced magnesium and calcium availability in soil solutions (Aber et al., 2001) and decreases in calcium foliar levels (Pilon et al., 1994). This may be due to a dilution effect resulting from high metabolism rates in roots during the winter months (Zogg et al., 1996) or the increase in sugar maple fine root mortality with an increase in soil temperature (Tierney et al., 2001).

Although these single factors of primarily natural orgins may lead directly to stress and possible decline of sugar maple, anthropologically induced alterations in soil nutrients resulting from pollution deposition may also contribute to sugar maple decline (Lovett 2000). Industry from the Great Lakes area as well as New York and Pennsylvania produces high levels of pollution, and the varied topography of the region tends to retain pollutant-laden clouds and fog longer than a flatter landscape might (Lovett 2000). The retention of this anthropomorphic pollution is most obvious in the Catskill Mountains, where significant damage due to acid rain is obvious mainly because of its centralized location around major pollutant production centers (Lovett 2000). The Catskill's proximity to New York City and its high concentration of automobiles explain much of the pollution effects seen in the mountains (Lovett 2000). Orographic uplift over peaks with a maximum altitude of 1300 meters also promotes the release of frequently acidic precipitation. Sugar

maple, with its sensitivity to drastic soil ionic imbalances, may be particularly susceptible to damage from this pollution deposition (St. Clair 2005).

The unique soil characteristics of the inceptisols in the Catskills make the region particularly susceptible to ion imbalances and pH changes, the two most influential results of pollution deposition (Tornes 1979). First, the parent material of this particular soil family is 40% mudstone and siltstone and 60% sandstone and conglomerates (Murdoch and Stoddard 1993), and bedrock with this composition is known to contribute a very low concentration of bases to daughter soils. These inceptisols are sandy loams containing an average of 60% sand, and exhibit exceptionally well-drained porous characteristics, allowing for easy leaching of nutrients (Kudich, 2000). Secondly, Catskill soils are acid-sensitive due to glaciation, which scoured exchangeable base-rich soils from ridgetops and slopes (Johnson 2000). Slope is a third suggested reason for poor CEC in these ridgetop soils, although correlations between these two measurements in field studies have yet to yield significant findings supporting this hypothesis (Johnston et al., 2000). Soil chemistry results show the mean of these soils the organic layer is 3.8, and the pH in the mineral soil averages to 4.3 (Lawrence 2000). This soil pH however varies with precipitation, however, which has been measured at pH levels as low as 2.1 (Driscoll, 2000).

These features of Catskill soils create a soil profile with poor ion exchange capacity, but anthropomorphic pollution additions to the ecosystem causes further problems with both CEC and pH. With less cation exchange taking place in these highly acidic soils, deposition promotes further leaching of nutrients and cations

already in low concentrations (Shortle 1988, Shortle 1992, Minocha 1988, DeHayes 1999, Driscoll 2001). In an example of this leaching, Catskill soils show spodic bands of accumulated metals showing that these compounds and valuable similarly-charged nutrients alike are leaching easily through these soil profiles (Lawrence, 2000). A study by Shortle in 1992 first indicated that soil base cations in the latter half of the 20th century were roughly half of those found before 1950, and atmospheric ion deposition is shown to be in strong correlation with the rate of ion loss.

The compounds mobilized in these soils are largely nutrients that are essential to plant growth. Nutrients such as phosphorus (Bernier and Brazeau 1988, Pare and Bernier 1989), potassium (Bernier and Brazeau 1988, , Ouimet and Fortin 1992), magnesium (Bernier and Braeau 1988, Horsley et al., 2000), and calcium (Heisey 1995, St. Clair 2004, Kobe et al., 2002) are found in small amounts in soils with average levels of acidity, but an influx of hydrogen ions from acidic precipitation mobilizes these nutrients and allows them to leach into the B horizons of the soil.

Of all micronutrient imbalances affecting sugar maple in this region, calcium concentrations in the soil have been hypothesized to have the most impact on its decline. This may be due to sugar maple's high uptake of this particular mineral (Dijkstra 2004). Calcium is an important element in that it both influences the growth of vegetation directly and regulates reactions with other elements in the soil solution. It is essential for the creation of root hairs, for the creation of calcium pectate, which helps to solidify cell walls, as an antioxidant, and creating permeable walls for the passage of minerals and water into the cell (Simon 1978).

Before the mid-1900s when acidic precipitation became more common, a forest soil deficient in calcium was a rare event (Shortle 1982, Wilde 1958). Calcium is normally found in the soil solution as a cation adsorbed to clay and other negatively charged surfaces. Calcium can be added to the soil in three ways: from the weathering of soil parent material, the decomposition of plant and animal tissues, and the dry deposition of dust, which contributes the largest proportion of calcium input of the three at any given time. Most of the calcium in the forest system, however, is bound to mineral substrates and therefore cannot be dissolved as a cation into the soil solution, making it unavailable for plant uptake even when it is present in the soil in large proportions (Brady 2002). The lack of calcium in forest soils is then attributed to the lack of calcium dust, the allocation of more calcium into biomass in mature forests, and the leaching of calcium with the assistance of anthropogenic deposition (Brady 2002, Hotopp 2002, Shortle 1982).

In reference to the idea that calcium is stored in biomass, some studies have shown that the decrease in calcium is not uniform across all stands of sugar maple, but instead suggest the usage and distribution of calcium is directly related to the age of the stand. A study done by Hotopp (2002) used snail shell calcium contents to find that sugar maple stands younger than 30 years had excesses in calcium but forests older than 30 years showed very low levels of calcium. He suggested that less available calcium in the older stands might have been the result of larger crowns with more foliage and woody biomass depletes more calcium from the soil. Further studies showed that the reduced calcium in the soils of older stands was also reflected in the leaf litter (Hamburg et al., 2003).

The leaching of calcium through the soil profile is the best supported hypothesis for calcium loss. The middle to high elevation slopes dominated by sugar maple in the Catskill Mountains are losing calcium faster than lower elevations and level ground due to the sandy, well-drained conditions of the inceptisol soils in this area (Long et al., 1997). It has been shown through foliar analysis that sugar maple growing on high elevation sites have consistently lower levels of foliar calcium than those growing in lower elevation sites (Kolb and McCormick 1993, Likens 2006, Johnson et al., 2000). This is explained by water movement through sloped forest soil which has higher runoff and ground water mobility (McCormick 1993). These soils also often have shallower horizons especially at higher elevations, leaving less soil to retain calcium and water and causing an even more inhospitable environment for trees with high calcium needs like sugar maple (McCormick 1993).

Soil calcium concentration, however, has been steadily falling over the past century at rates that are not easily explained by natural causes (Johnson et al., 1994i, McLaughlin et al., 1999). Soil calcium decreased significantly from 1932 to 1984, and again at a faster rate from 1986 to 1990 (Johnson 1994i, 1994ii). McLaughlin (1999) has shown that reduced growth in red spruce is correlated with reduction in available calcium, and later studies have shown this calcium decrease is due primarily to acid deposition (Markewitz et al., 1998). Ice core studies have also shown that there is a marked decrease in the amount of calcium dust present in our atmosphere over the past few decades compared to levels found for the past 20,000 years, suggesting that acid rain is not the only cause for the drop in calcium in forest soils but could still be good reason for changes in forest calcium balances (Mayewski

1990). Finally, strontium studies have shown calcium budgets in both New Hampshire and New York have been shown to have significantly decreased stores of calcium available to plants in Northeastern forests (Miller et al., 1993, Bailey et al., 1996).

Despite its general declining concentration, the amount of calcium in the soil is not only important as a nutrient but also to buffer other compounds that may be harmful for sugar maple tissues. As calcium becomes exchangeable throughout the organic and A horizons of the soil, calcium helps to raise the pH of the soil solution by neutralizing the charge of hydrogen ions and counteracting the effect of acid rain. As nitrate and sulfate from acid deposition move through the soil, calcium leaching is enhanced and soils may become calcium deficient (Brady 2002).

Sugar maple is not an opportunistic species, so specific ion imbalances and other soil changes such as aluminum and pH as severe as what is seen in the Catskills have strong biological consequences. Since more than half of the forest's available nutrients (and the fine roots that absorb them) lie within the organic horizon of the soil column, even a little leaching is too far for most roots to access (Fahey and Hughes, 1994). The interaction between oxidative stressors and nutrient imbalances clearly plays a very strong role in the decline of this species (Bertrand 1994, Horsley et al., 2002).

Specifically, a limited pool of phosphorus limits the production of ATP, ADP and nucleic acids, all elements of growth that are essential to the production of new tissues. Lack of phosphorus leads to stunted meristematic growth, seed production, and photosynthetic efficiency (Horsley, 2000). Phosphorus limitations may have led

to the reduced photosynthetic efficiency as was seen in St. Clair's work (2004), where both canopy trees and seedlings increase concentrations of antioxidant compounds with nutrient imbalances in the foliage. Magnesium is responsible for carrying phosphorus within the plant, the formation of sugar, oil, and fats, and is an enzyme activator. Magnesium is also the central element of chlorophyll, and so a lack of this element limits carbon assimilation (Bernier, 1988). Potassium is a structural component of DNA, enzymes, and proteins, and the lack of any of these greatly reduces the rate of respiration and photosynthesis. Potassium deficiencies also presents itself with poor stem growth and seed production (Wilmot et al., 1996, Horsley et al. 1997).

The secondary effects of these ion imbalances, especially calcium, also have extensive consequences. The uptake of nutrients in sugar maple, especially nutrients found in smaller concentrations due to the leaching described above, is much more efficient with the help of arbuscular mycorrhizae (Heijne et al., 1996, Clark 1997, Tinker et al., 1992, Clark and Zeno 2000). However, the mycorrhizal species that sugar maple depends on are sensitive to soil acidification and shows restricted uptake in acidic soils (Hepper 1984, St Clair 2004, Heinje 1996, Clark 1996, Smith 1997), so the reduced efficiencies of these species could jeopardize the uptake of nutrients by sugar maple. In addition to low overall efficiency, mycorrhizal fungi also show low colonization in *Acer* species at low soil pH (Ouimet et al., 1995, Coughlan et al., 2000, Frankland and Harrison 1985). With sugar maple's heavy dependence on mycorrhizae for nutrient uptake, the detrimental effects of acid precipitation and the acidic soil solutions it creates is a concern.

Nutrient stress also has positive interactive effects on browsing. These events deplete carbohydrate stores, the main value assessed when determining sugar maple health (Wargo et al., 1997). Since calcium and magnesium dichotomies affect carbohydrate stores as well (Wargo et al., 2002), it is very possible that the combined effect of calcium deficiencies, magnesium deficiencies, and herbivory deplete carbohydrate reserves interactively and affect sugar maple growth negatively (Horsley et al., 2000). Concentrations of micronutrients in the foliage have a direct effect on herbivory rates, and leaves with greater concentrations of micronutrients and nitrogen are preferred by insect pests and are browsed more repeatedly than poor nutritional content leaves. Since sugar maple foliage tends to have a high nitrogen concentration in comparison to other species in this region, this repeated herbivory leads to sugar maple decline (Horsley 2000, Tripler 2004). This rate of defoliation is crucial, as an increase in insect browsing also increases the rate of defense compound production and other tactics to prevent further herbivory (Glynn et al., 2003).

Soil acidification may also lead to increased mobility of potentially toxic compounds that are usually kept in low concentrations. As more hydrogen ions are present in the soil, immobilized aluminum becomes mobile and enters the soil solution thereby potentially accumulating in plant tissues. High aluminum levels in plant tissues may prevent cell division and reduce overall root growth (Kobe et al., 2002, Mohamed et al., 1997) and may also impede the intercellular movement of phosphorus, magnesium, and most importantly, calcium (Dobermann and Fairhurst 2000). While sugar maple root tissues are already in stress from soil acidity, the added influence of aluminum ions can have deleterious effects. When aluminum

accumulates in sugar maple to the point of necrosis, this over-abundance is termed "aluminum toxicity" and can cause sugar maple dieback, necrotic spots, lack of frost/drought tolerance, and in extreme cases, tree mortality (Sverdrup 1994).

Aluminum levels are not only damaging to sugar maple tissues directly, but also affects the efficiency and vitality of the mycorrhizal populations sugar maple depends upon. While mycorrhizae help to ameliorate the uptake of aluminum to a point (Clark and Zeto 2000), acidic, aluminum-heavy soils where Acer vitality is most compromised, hyphal growth, spore production and colonization is at its lowest (Zahka et al., 1995, Bartolomeesteban and Schenck 1994. This leaves sugar maple in a nutrient-deficient soil with reduced mycorrhizae to aid in the absorption of already-scarce nutrients. Aluminum can also decrease defense-chemical production for disease and herbivorous protection (Clijsters et al., 1999). These studies show that increases in aluminum in the soil solution has negative effects on sugar maple through numerous pathways. While aluminum in high concentrations can be damaging to sugar maple health, higher concentrations of magnesium and calcium minimize aluminum's negative effects by competing with it for absorption and binding sites or by increasing fine root growth (Long et al., 1997, Kobe 2000). Of course, the very conditions that allow aluminum to be so prevalent in the soil are the same conditions that promote ion leaching and cause lower levels of calcium and magnesium.

In addition to impacts on soil calcium, acidic deposition is also having a major impact on the concentration of the macronutrient nitrogen. As the essential component for all amino acids and chlorophyll, nitrogen is directly linked to the

growth and productivity of the plant. Secondary metabolites produced by the plant for defense mechanisms against disease, pigmentation, allelopathy, and other functions also use nitrogen as a large component of their molecules. Nitrogen is found in the forest ecosystem in numerous forms, but its processes are regulated by rates of mineralization and immobilization. The concentrations of ammonium, nitrate, and other nitrogen species is dependent on a number of factors including soil organic matter, total phosphorus, soil temperature and pH. Increases in ammonium nitrate in the sol can increase denitrification (Ashby et al., 1998). Since nitrifying activity is enhanced at lower the soil pH, nitrate is found in higher concentrations than ammonium in those soils (Ashby et al., 1998). Since sugar maple prefers to take up nitrate as opposed to ammonium, this process increases its growth rate (Templar 2004). However, this may also lead to limitations with other nutrients, especially calcium, so the overabundance of N may lead to stress as well (Aber 1999).

Nitrogen is commonly thought to be the limiting factor to plant growth in forest systems. However, recent nitrogen deposition has altered nitrogen from a limiting nutrient to a nutrient in surplus in some areas such as near industrial cities and metropolises have extremely high levels of nitrogen (Lutz 1946, Aber 1989, Driscoll et al., 2001). In these forests, much like temperate ecosystems around the globe, nitrogen in its various forms has become so abundant that these areas have been termed “nitrogen saturated’ (Driscoll et al., 2001). Researchers have not yet agreed on a full set of defining factors of nitrogen saturation, though a number of characteristics are universal to the various explanations presented by various studies. It is clear that a surplus of nitrogen damages fine roots, mycorrhizae, and sensitive but

essential microbial communities (Binkey and Hogberg 1997). However this situation is difficult to diagnose because it is extremely difficult to quantify nitrogen leaching and denitrifying processes, major parts of the nitrogen saturated diagnosis (Hogberg et al., 2000). The most common diagnostic key for determining nitrogen saturated is through increases in streamwater nitrate concentrations (Vitosuek 1997). Not only is this indicative of an ionic imbalance in the soil, but the acidification of streams can have even farther-reaching implications as fish-bearing waterways and metropolitan reservoirs become acidified (Newell 1993).

Despite the lack incongruity of the definition of nitrogen saturation, studies have repeatedly found that the Catskill Mountains fit whatever definition is being used (Stoddard 1994, Lawrence 2000, Lovett 2004). Once acidic and nitrogen-laden soil solution reaches the base-poor parent material of which the Catskill soil consists, previously acidic soils and soil water with poor cation-exchange capacity become even more acidic (Bricker and Rice 1989, Johnson 2000). Nitrogen retention in the Catskills ranges from 49 to 90% of the atmospheric input, and this shift from 49 to 90% in the chemical makeup can be drastic on the soil nutrient balance (Lovett et al., 2000). As stated before, with sugar maple's sensitivity to base ion concentrations, this imbalance can deleteriously affect this species in numerous ways (Lovett et al., 2000).

Other issues resulting from nitrogen saturation is the presence of a deep organic soil horizon, which indicates a lack of microbial communities to break down humus. Their absence is thought to be due to the increased acidity of the soil due to excessive nitrogenous deposition (Vitsouek 2000). Foliage with higher

concentrations of nitrogen also spurs higher growth rates of Lepidopterans, Acer's chief defoliator (Kinney et al., 1997), creating a vicious cycle of herbivory that may compound environmental stress on sugar maple.

With these observations of decline linked to calcium and nitrogen concentrations, a number of experimental studies have been conducted. The response of sugar maple to calcium concentration has been observed with the artificial addition of calcium amendments as lime, dolomitic limestone, or wollstonite. Since declining trees store glucose and fructose in their roots instead of less-soluble starch compounds as healthy trees do, the presence of high levels of starch in sugar maple roots in response to liming suggests that adding calcium increases sugar maple health (Wargo et al., 2002). One concern with a number of these amendment studies is the use of calcium carbonate or calcium oxide, which may confound the effects of calcium additions with pH alterations (Long et al., 1997). Previous studies have shown that calcium additions to sugar maple increases leaf area, fine twig growth and seed production as well as a reduction in stressors (Long et al., 2000, Long et al., 1997, Liu et al., 1994, Moore et al., 2002). However, calcium additions to other species have not resulted in increased growth, suggestion that sugar maple is a particularly sensitive species to base ion concentrations (Long et al., 1997, Kobe 2000).

Increased soil nitrogen treatments are not as well studied as calcium additions, though some studies have been conducted. Many nitrogen addition studies conducted over short periods of time have failed to show an effect on its decline. For example, St Clair found that Pennsylvanian sugar maple photosynthetic efficiency showed no

correlation with nitrogen foliar content (St Clair 2004). Also, disease and decline did not show a correlation to soil nitrogen concentrations in Pennsylvanian stands either (Horsley 2000, Catovsky al 2002, Duchesne et al., 2002). Some studies conducted over longer periods of time have shown increases in growth due to nitrogen additions (Ellsworth 1998, Binkley and Reid 1984). Sugar maple dominated forests in northern Vermont fertilized with twice the ambient total annual nitrogen deposition amount resulted in an increase in growth (Ellsworth 1998).

It is important to note, however, that these findings are not in contrast with the theory that additional nitrogen can be detrimental to sugar maple health, since all of these studies were conducted on soils that were known to be either nitrogen-poor or at a normal level of nitrogen. The only study that has been conducted on nitrogen additions which mimic the effect of nitrogen saturation is Magill (2000), and that study differs in that it was a long-term study as opposed to those stated above. For the first six years, sugar maples treated with nitrogen additions showed signs of increased biomass and vitality. However, after six years, sugar maples began to decline steadily. This pattern of increases in sugar maple growth followed by quick decline is likely the sort of observations one would expect to see in the nitrogen saturated soils of the Catskill Mountains.

Though many factors are at play in the decline of sugar maple, one of the simplest ways of testing the importance of the imbalance of nutrients in the soil seems to be through the addition of these nutrients. Since high N levels can lead to loss of Ca, the interaction of these two nutrients may be especially important to study. While one study has focused on the effect of calcium and nitrogen together (Ellsworth and

Liu 1994), there has been no work on the addition of both calcium and nitrogen to Southern New York soils. Since Ellsworth and Liu (1994) and Liu's further study (1997) found calcium and nitrogen together may limit photosynthesis in Vermont, the exploration of calcium, nitrogen, and both calcium and nitrogen amendments on sugar maple health is essential for understanding the vitality of this species and, perhaps, to develop a way to improve the health of these stands.

Therefore the purpose of this study was to examine the response of sugar maple in New York State's Catskill Mountains to calcium and nitrogen addition to the soil. The effects of soil amendments with calcium and nitrogen on the vitality of sugar maple were assessed in two ways: through the measurement of photosynthesis using an infrared gas analysis instrument (IRGA), and through the measurement of respiration by analyzing heat evolution with microcalorimetry. We also determine whether measurement of photosynthetic carbon assimilation measurements taken though gas exchange analysis could be correlated with measurements of microcalorimetry. The hypothesis tested was that seedlings and mature trees would show increased carbon assimilation and anabolic metabolism in response to calcium soil amendments and a reduced photosynthetic response to nitrogen addition if the soil was already nitrogen saturated.

Chapter 2: Methods

Site Description

The Catskills Mountains, a 3700 km^2 area in Southern New York, is dominated by a sugar maple-American beech-yellow birch forest with Eastern hemlock in rare pockets (Fig. 2.1a) (Lovett 2000, Horsley 1997). The annual average temperature is 4.3◦C with an annual precipitation of 150 cm, 20% of which is snowfall (Lovett 2000). The soil parent material is mostly heavily eroded sedimentary rocks consisting of 60% sandstone and 40% siltstone and mudstone (Stoddard and Murcdoch 1991). The study site (41◦59'lat., 74◦28'long.), which was clear-cut 65 years ago, is on the northwesterly slope of Wildcat Mountain with a maximum elevation of 1238 meters (Fig. 2.1b).

Experimental Design

The study was a randomized complete block design with repeated measures. The sampling scheme was incomplete instead of complete due to two complications: gas analysis of seedlings was interrupted during one sampling period due to equipment malfunction, a lack of seedlings in one plot and difficulty in obtaining samples for foliar analysis for some mature sugar maples that were too tall to sample.

In the fall of 2003, twelve plots within 3 blocks of varying elevation and slope were fertilized either with dolomite, ammonium, both, or left untreated as a control (Fig. 2.1b). Each plot measured 50 by 50 meters square and was protected from

a.

b.

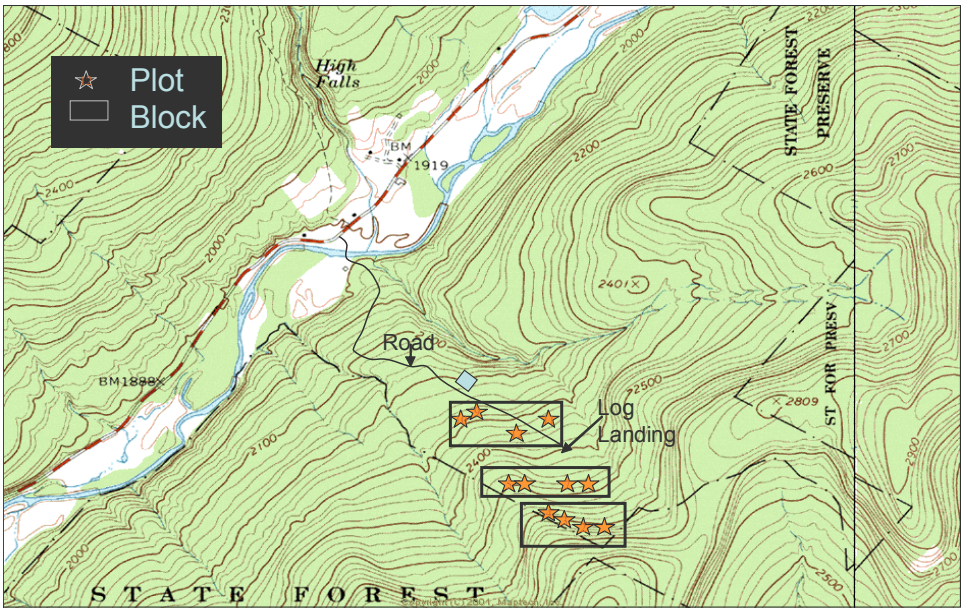


Fig. 2.1.a. Overview of the location of Wildcat Mountain within Frost Valley YMCA camp (Google Earth DirectX 8, 2007).

b. Location and topography of Wildcat Mountain, Ulster County, New York State. Stars indicate plot locations; each rectangle indicates a block. Treatments were randomly assigned to each plot within each block.

herbivory by a nine-foot plastic fence. Blocking for slope was essential since nutrient declines typically worsen on upper slopes (Towers 1984). Plots treated with calcium additions were treated once with dolomitic limestone with a ratio of 2:1 of calcium to magnesium. 9080 kilograms of dolomite per hectare was deposited in pellet form. Plots receiving nitrogen additions were sprayed annually with 25 kilograms per hectare of ammonium nitrate using a backpack sprayer. was deposited, This application was roughly twice the ambient input (Lawrence, G., USGS, personal communication).

Field Methods

Soil Sampling

Soil was collected in May of 2007 using the methods outlined by Johnson (2000). Two samples were collected from each treatment plot. Each sample consisted of an equal mixture of soil harvested from the mineral horizon of three pits 10 cm in diameter and ranged in depth from 3 to 10 cm deep. The mineral horizon was determined through visual color analysis, the presence of the fine root mat in the layer directly above it, and the absence of humus or other organic debris. Care was taken to ensure that no soil from the O horizon was included in the sample. Samples were deposited in sealed plastic bags, returned to the lab, and allowed to air dry on newspapers. Additional samples were collected in the same manner to replace some samples that were damaged during shipment to the soil testing laboratory in early June of 2007.

After drying, soil samples were sifted using a 20 mesh sieve and packaged in air-tight plastic bags and shipped to Pennsylvania State University's Agricultural

Analytical Services Lab. Soil was measured for the following: water pH, phosphorus concentration, potassium concentration, magnesium concentration, calcium concentration, total nitrogen concentration through combustion, aluminum concentration, and cation exchange capacity (CEC).

Soil pH was determined with the 1:1 water:soil method (Eckhert and Simms 1995). Phosphorus, magnesium and calcium were determined with the Mehlich 3 (ICP) (Wolf and Beegle 1995). Soil calcium and aluminum concentrations were found through a 0.01M $SrCl_2$ extract. Soil nitrogen was determined by combustion and given as a percentage of dry weight.

Foliar Sampling

Foliage samples for analysis were harvested in May of 2007 from mature trees only to prevent negative impact on seedling regeneration. All foliage was harvested with the use of a pole-pruner, and due to equipment limitations all foliage samples were collected from the base of sugar maple crowns. Three trees were sampled in each plot of randomly selected size, age, and position in the plot considering the limiting factor of the height of the pole-pruner. Randomness was ensured through a modified form of simple stratified random sampling. Three numbers were selected at random from the page numbers of a field guide. Each number selected indicated the number of steps the sampler must take from the gate of each plot in a single direction into the plot, and once at that location, the closest sugar maple was selected for sampling. Each sample consisted of subsamples consisting of no less than five leaves from at least two different areas of the crown. These subsamples were represented in each sample equally in number. Leaves were immediately placed in air-depleted, air-

tight plastic bags and placed in a dark cooler ranging in temperature from 2 to 6 ◦ C for transport to the laboratory where they were stored in a refrigerator at 4 ◦ C.

Leaves were then dried in a drying oven at 70◦ C for one week at in paper sacks. Petioles were removed and blades were first ground in a mortar, then ground in a modified coffee bean-grinder to a less than 20 mesh grind. Ground leaf matter of a known mass was then placed in tin ampoules and brought to the University of Maryland's Maryland Soil Testing Laboratory for analysis using a CHN-600 Elemental Analyzer System (LECO Corporation: St. Joseph, MO). Carbon, hydrogen, and nitrogen concentrations were expressed in percent of the total mass of the sample.

Photosynthetic Measurements

Photosynthesis was measured as net CO_2 uptake using the Li-6400 Portable Photosynthesis System (Licor Biosciences, Lincoln, NE) that utilizes an infrared gas analyzer in an open design system to determine photosynthetic rates as a function of carbon dioxide uptake. The open system design maintained a constant flow of air through a leaf chamber to minimize the effect of the enclosed chamber on the measured values. A thermocouple allowed the recording of both leaf and ambient air temperature and a red-blue LED light source allowed for the leaf to be exposed to varying PAR levels.

Three sugar maple seedlings of three or four years of age were sampled in the field in each of the 12 plots on the site. These seedlings were randomly selected at least a distance of 3 meters from the edge of each plot to reduce treatment edge effect.

Random sampling, again, was conducted with a modified stratified randomized sampling scheme: randomly chosen numbers determined the number of steps taken from the gate of each plot, and the closest sugar maple from the end point was selected for sampling. However, this method was only utilized for five of the twelve plots since seedlings were scarce in most treatment plots. In this case, the first four seedlings found were selected for sampling. One plot had no seedlings at all in the growing season of 2006. Measurements were taken three times: June 2005, July 2005, and June 2006 over a period of four or five days between the hours of 9 am and 4 pm.

Each seedling was held in complete darkness for two minutes before measurements began to allow for acclimation before the recording of relative dark respiration. Humidity and gas concentrations were held at ambient levels, while temperature was held at the average predicted midday temperature for each sampling day. Incoming air was brought into the chamber through a 2 meter-long piece of tubing to prevent the affect of the researcher's respiration. Photosynthesis light response curves were obtained by varying the incoming photosynthetically active radiation (PAR), emitted from artificial quartz red and blue light emitting diodes and the system was preprogrammed to take a set of measurements at a range of incoming PAR values of 0, 100, 150, 200, 250, 300, and 500 $\mu E\ m^{-2}s^{-1}$. The rates of net CO_2 assimilation (A: $\mu mol\ m^{-2}\ s^{-1}$), stomatal conductance of water vapor (gs: $mol\ m^{-2}\ s^{-1}$) and transpiration ($mmol\ m^{-2}\ s^{-1}$) were calculated by the LI-6400 data analysis program which utilizes the Von Caemmerer and Farquhar (1981) general gas exchange formula.

Similar measurements were made on harvested mature leaves which were obtained by shooting shoot tips down with shotgun. These samples were stored in air-tight sealed bags and placed in a cooler below five degrees Celsius during transport to the lab and measured over a period of 3 days. Leaves with minimal shotgun pellet or insect damage were selected for measurements of gas exchange as described above, and microcalorimetry (see following section). A trial was run to ensure the tissues sampled were not affected by this storage. No significant difference between sampling days was found ($P > 0.05$), indicating this was a suitable storage method which did not affect measurements (Fig 2.2).

Four elements of the light response curves were analyzed: respiration in darkness, the light compensation point, the light saturation point, and apparent quantum efficiency (AQE). Light compensation point was defined as the light level at which net assimilation was equal to respiration. Light saturation was defined as the point where the slope of the light response curve reached 10% and apparent quantum efficiency (AQE) as the slope of the light response curve between 0 and 100 $\mu mol\ m^{-2}\ s^{-1}$ PAR.

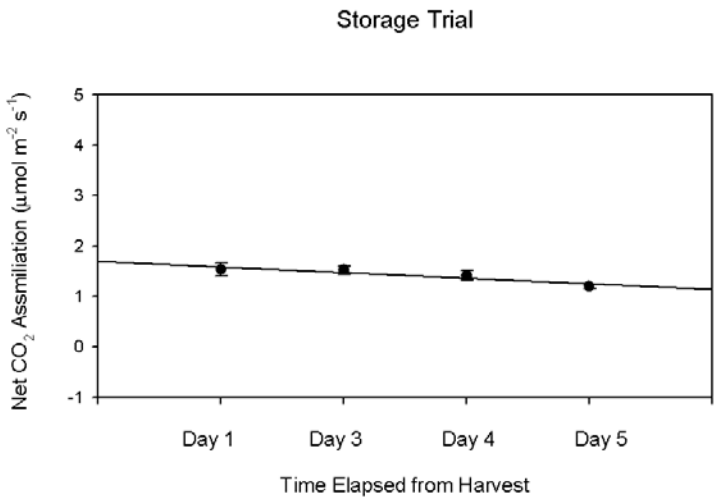


Fig. 2.2. Net assimilation (A) of leaf sample stored from 1 to 5 days following collection. No measurements were taken on day 2 to mimic a day of travel as was done in the field. No significant deviations from the mean ($P<0.05$) were found.

Microcalorimetry

Overview

So-called dark respiration rate has traditionally been measured using an infrared gas analyzer with a darkened leaf chamber. This, however, may only account for carbon dioxide evolution and a variety of other processes may never be seen as carbon dioxide production (Criddle and Hansen 1991). For this reason this study measured respiration using microcalorimetry, a much more precise method for measuring the entire leaf's metabolism (Criddle and Hansen 1991).

Respiration and its two components - catabolism (the energy-yielding processes such as the Krebs cycle and glycolysis) and anabolism (the utilization of energy to create cellular components, photosynthate, and larger molecules) - are the sum of all of the chemical reactions that occur within the plant, and the ratio of these functions can show whether the individual plant is in decline or creating new biomass. With the use of a microcalorimeter, the production of heat and carbon dioxide per unit of sample leaf tissue can be precisely measured. These measurements can then be compared through calculations derived from Criddle (1991) and Hansen's (1992) past work to estimate the balance between energy of catabolism and anabolism.

Calorimetric Theory

The oxidation-focused method outlined in Criddle (1991) and Hansen (1992) is the basis of this study. The general theory supposes that the rate of respiration multiplied by the efficiency controls the amount of energy available for growth, and by finding these values as a function of temperature and comparing, in this case, tissues treated with differing nutrient treatments, stress can be quantified (Hansen et al., 2002). Since the reactions of cellular metabolism produce heat, it is possible to measure temperature changes in a chamber enclosing the tissue under various conditions to infer conclusions about plant biochemical processes. Heat rates are proportional to reaction rates, which are proportional to the rates of growth and processes within the tissue (Criddle 1998). The equation used to predict the amount of energy available for growth, or total anabolic activity, is as follows:

$$R_{SG} \Delta H_B = - \Phi - R_{CO2} (1 - \gamma p/4) \Delta H_{O2}$$

$$R_{CO2} = A_{CO2} e^{-\mu CO2/T}$$

Where ΔH_B is the change in enthalpy per mol of carbon (or the change of substrate into structural biomass), γp is the average oxidation state of the substrate carbon (which, in the case of carbohydrate, is 0), μ is the temperature coefficient and ΔH_{O2}, the constant from Thorton's Rule, shows the heat of combustion of organic compounds expressed per mole of oxygen (Hansen and Criddle 2005). Φ is the rate of energy loss to the surrounding environment. The resulting $R_{SG} \Delta H_B$ is considered a measure of energy available for growth. ΔH_B, the enthalpy change, is the result from this reaction:

$$\Delta H_B: \ C_{photosunthate} + (N, P, K, etc.) \rightarrow C_{biomass} + xO_2$$

Which illustrates the difference in the heats of combustion per mole of carbon when used for the production of biomass as opposed to the breakdown of photosynthate. For these measurements, ΔH_B is assumed as +50 kJ $mole^{-1}$ (Hansen 2000)

$$\Phi = A\Phi_e^{-\mu\Phi/T}$$

This equation uses A, a constant, to help derive R_{CO2}.

The respiration parameters measured and calculated for analysis were the calorimetric respiration ratio, or CO_2 production rate (R_{CO2} µmol mg-1 s-1) per total metabolic heat produced (Rq), heat lost per mole of carbon dioxide formed (q/R_{CO2}), metabolic heat rate (q, µW mg-1), the temperature coefficient of metabolic heat rate

(μq, kiloKelvin) and the calculated specific growth (R_{sg}, μmol C mg^{-1} s^{-1}) (Hansen and Criddle 2005).

Harvesting Methods

For respiration measurements, mature foliage was shot with a shotgun and lead buckshot from the canopy twice during the summer of 2005 and three times during the summer of 2006. Foliage was collected from seedlings twice in the summer of 2005 and once in the summer of 2006. Three mature and three seedling foliage subsamples were harvested from each plot. The foliage was then immediately encased in air-tight plastic bags and placed in darkness inside a cooler. The foliage was kept at temperatures between 1 and 5 degrees Celsius during transportation to the laboratory. Once in the laboratory, the foliage was be kept between 1 and 5 degrees Celsius in a refrigerator. Care was taken to keep the foliage in near-darkness before in-lab measurements were taken. Due to the limited number of seedlings present a seedling from each plot was harvested each summer. In 2006, some seedling calorimetric and photosynthetic data is missing due to this lack of seedlings.

Laboratory methods

After selecting for leaves with minimal insect and shotgun damage, a single leaf from each branch was randomly selected for calorimetry measurements. All leaves were allowed to equilibrate to room temperature for twenty minutes before either measurement began. Foliage selected to be tested via calorimetry was excised from the branch immediately after removal from the refrigerator. Each leaf was

excised from the petiole at the leaf base with a razor blade. Each leaf was patted dry with a laboratory tissue to prevent evaporation from interfering with the signal. In the event the leaf was too large for the ampule, the leaf was cut with a clean, dry razor blade and intravenous tissue was used – approximately 150 mg fresh weight of tissue was used for each measurement. Leaves were allowed to equilibrate to ambient humidity levels for 10 minutes in near darkness before calorimetric measurements began.

Foliar tissue was then placed in an ampule of the calorimeter and care was taken to prevent the creasing or damage of the leaf (Illus. 4.1). Calorimetric data were taken on a multi-cell differential scanning calorimeter (Calorimetry Sciences Corporation, Lindon, UT), allowing for three samples to be run at once. Samples were staggered by treatment and blocked by day to reduce systematic errors and any effect of storage time or time of day. All measurements were taken in isothermal mode at the average midday temperature at which they were harvested. A stream of pure oxygen prevented water condensation on the cells when data points were taken at a temperature differing from room temperature. The ampule, chamber and sample were then allowed to reach thermal equilibrium for ten minutes before any measurements were taken. This first heat measurement, Rq, gives a value related to total metabolism. Next, a vial containing 50 µL of 0.4 M freshly prepared sodium hydroxide was quickly inserted into the ampule. Heat production from the sample metabolism as well as the heat production from the formation of carbonate was recorded after a ten minute equilibration period. Rq subtracted from this value gives the heat of carbon dioxide production, or $R_{CO}2$ (Criddle and Hansen 1999). A final

measurement of Rq was made after the sodium hydroxide vial had been removed. The two Rq measurements were averaged. After measurement, all samples were dried for 4 days in a drying oven at 70◦ C and mass for each leaf sample was determined, an essential component of the Criddle/Hansen calorimetric calculations (Fig 2.3).

Trials were conducted to ensure that the delay in analysis did not affect data points. Sugar maple foliage was harvested in imitation of field measurements and then stored in a cooler for a day before placing in a refrigerator. Measurements were taken at one, two and three days past harvesting (Fig . 2.4). No significant difference in qMet data points was found ($P>0.05$).

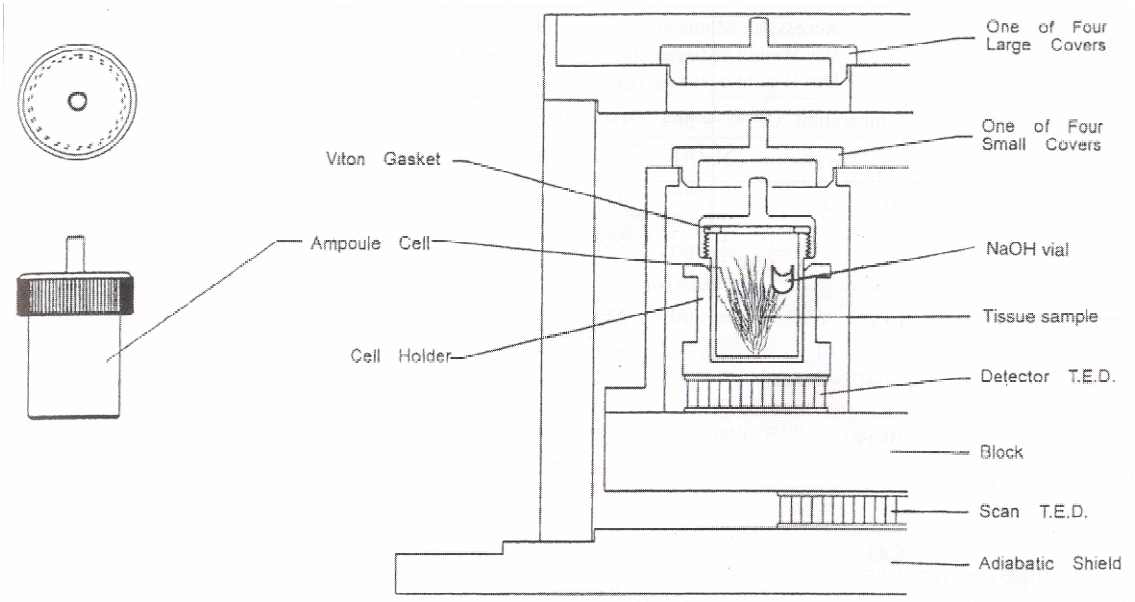


Fig. 2.3. Diagram of ampoule within a differential scanning microcalorimeter, showing the vial placed within the ampoule with the sample when measuring qNaOH (Hansen and Criddle, 2005).

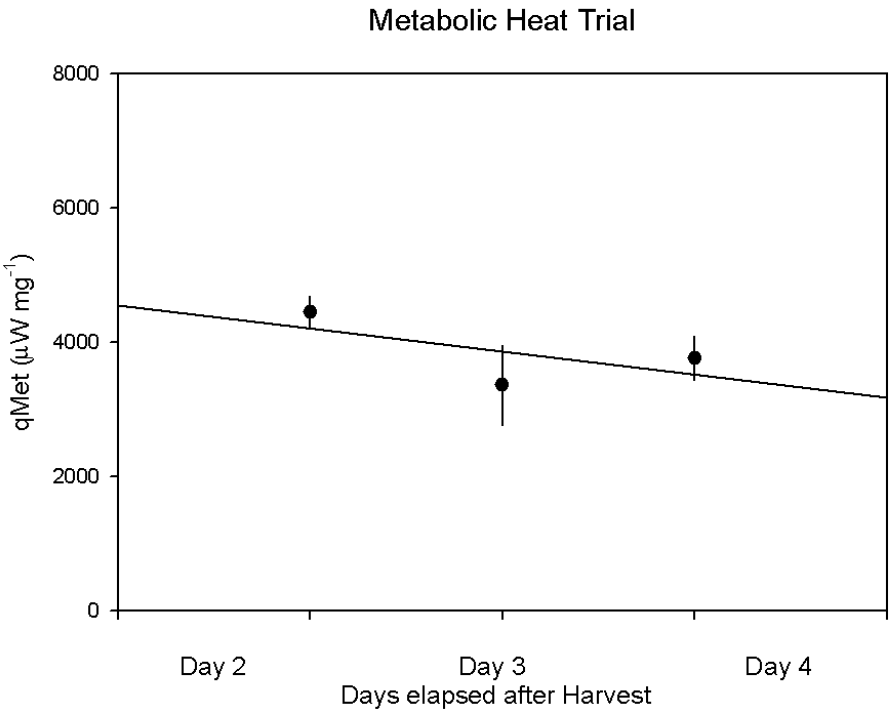


Fig. 2.4. Measurements of qMet for sugar maple leaves collected and stored for a four day period. Each point is the mean of samples on days 2-4 following collection. This storage trial showed no significant difference in measurements taken immediately or after an imitation of storage methods used in this study ($P \leq 0.05$). Measurements were not taken the day after harvest to mimic the day of travel used in field collection.

Statistical Analysis

The experimental design of this study was a randomized complete block design utilizing repeated measures (Dutilleul 1993, Kristensen and Hansen 2004). The design was determined to be incomplete due to a mechanical malfunction during one of the field sampling periods and the lack of seedlings in one plot. Data were analyzed using a mixed effect analysis of variance model with PROC MIXED with a blocking factor of slope/elevation effects in plot location with time of year (repeated measures) as split plot. Soil amendments were considered as a single independent variable for all measurements. Statistical significance was determined as $\alpha \leq 0.05$. SAS software version 9.01.01 was used for the analysis (SAS 2003). Fisher's least significant mean comparison test was used for means separation.

Chapter 3: Results

Chemical Analysis

Soil Analysis

Overall the soil amendments had a significant effect on soil pH, Ca, N and Al but did not significantly alter P, K, Mg, Cation Exchange Capacity (CEC) or the ratio of Ca to Al (Table 3.1). In particular, calcium amendments increased the soil pH ($p < 0.001$) and calcium levels in the soil ($P < 0.05$) (Table 3.1). The untreated soils were very acidic (pH = 3.6), and in comparison, the calcium treated soils showed a mean increase in pH to 4.8, over a tenfold magnitude increase ($P < 0.001$). Soil N concentration show trends toward reduction while CEC showed a trend toward an increase (1.52 to 3.87 mg/kg^{-1}, P = 0.06) in the Ca treated plots but these were not significantly different.

The addition of N to the plots resulted in little measurable change in soil chemistry. In terms of absolute values, nitrogen fertilization increased soil N levels from 9.3 to 16.8% of dry weight, although the difference was only statistically significant between these at P= 0.07. There was no significant effect of N addition on soil pH, calcium concentration, or aluminum level compared with control plots.

Simultaneous application of N and Ca led to several interactions in terms of soil chemistry. While soil pH and Ca levels responded similarly to Ca application alone, the application of N in concert with Ca did not increases soil N levels (Table 3.1). In fact soil N levels in these plots were reduced compared to the supplemental

N and the control plots. It is not clear whether this difference was due to enhanced uptake by the plants in the presence of higher Ca levels or to soil physical or biological processes. CEC of the soil more than doubled in the Ca treated plots with or without the addition of N but this was not significant due to the low sample size and high degree of variability. Likewise Al levels in the soil increased with the addition of Ca and N but this was not significant with either nutrient alone. However a strong trend was noted in the Ca treatment so again sample size may have contributed to lack of statistical significance observed.

Table 3.1. Soil nutrient characteristics from 3 replicate blocks of 4 soil treatments of plots in the Catskill Mountains of New York. Each value is the mean and standard error (in parentheses) of 2 subsamples from each plot. * Denotes $P < 0.05$, ** denotes $P < 0.001$ for overall treatment effects. Values in columns followed by the same letter were not significantly different according to Fishers multiple mean comparison test.

Trt	**pH ****	**Ca (mg/kg^{-1}) ***	**P (mg/kg^{-1})**	**K (mg/kg^{-1})**	**Mg (mg/kg^{-1})**	**CEC ($mg\ kg^{-1}$)**	**Al (mg/kg^{-1}) ***	**Total Nitrogen (% by weight) ***
Control	3.7 (0.21) b	310 (187) b	12.6 (2.0)	17.8 (4.5)	276 (261)	1.5 (2.61)	50.3 (1.3) b	9.3 (5.4) ab
Ca	4.8 (0.10) a	1475 (300) a	12.1 (4.9)	13.8 (6.9)	300 (210)	3.9 (2.97)	57.8 (2.0) ab	2.7 (1.0) ab
N	3.6 (0.33) b	200 (226) b	14.8 (3.2)	18.6 (5.1)	366 (284)	1.0 (2.98)	48.2 (1.2) b	16.8(3.2) a
Ca + N	4.7 (0.21) a	1385 (194) ab	9.38 (8.5)	13.7 (4.9)	500 (253)	3.7 (2.86)	58.9 (1.7) a	2.5(7.8) b

Foliar Analysis

Application of both Ca and N tended to increase foliar N levels but surprisingly this was only significant in the plots that received supplemental Ca as nitrogen levels of the foliage were unaffected by nitrogen fertilization. No other significant differences were seen in foliar concentrations of carbon, hydrogen or the C:N ratio (Table 3.2).

Table 3.2. Foliage nutrient characteristics from 3 replicate blocks of 4 soil treatments of plots in the Catskill Mountains of New York. Each value is the mean and standard error (in parentheses) of 2 subsamples from each plot. * Denotes $P < 0.05$ for overall treatment effects. Values in columns followed by the same letter were not significantly different according to Fishers multiple mean comparison test.

Treatment	N *	Carbon	C:N	H
Control	2.99 (0.32) b	46.0 (0.2)	15.9 (0.64)	3.68 (0.2)
Ca	3.57 (0.38) a	46.3 (0.3)	13.0 (0.11)	4.80 (0.3)
N	3.39 (0.38) ab	46.5 (0.02)	13.5 (0.07)	3.60 (0.3)
Ca + N	3.18 (0.39) ab	45.65 (0.1)	14.3 (0.39)	4.72 (0.2)

Photosynthesis

Light Curves

Although no statistical analysis was conducted on the light curves as a whole, points within the curves were analyzed below (Fig 3.1). In general the light response curves show some seasonal differences and suggest that these seedlings may have

adapted through the season to increased shading beneath the canopy. Late May/June in 2005 and 2006 showed very similar responses to all treatments although the calcium treated seedlings appeared to have a higher saturated assimilation rate on

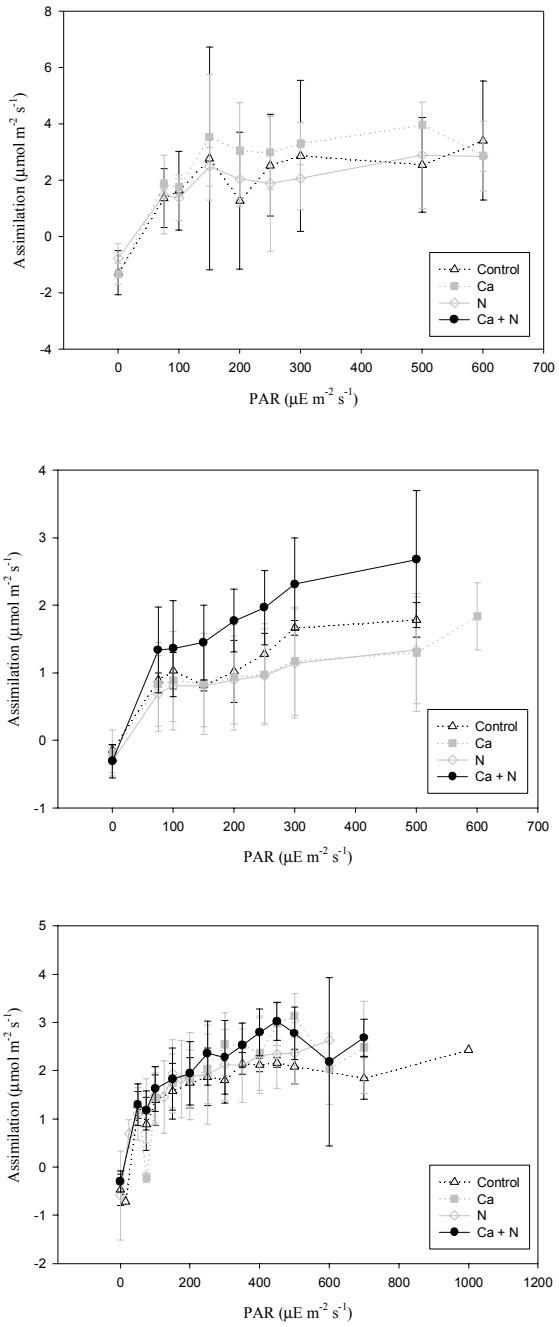


Fig. 3.1. Light response curves for early summer 2005 (upper), late summer 2005 (middle), and early summer 2006 (lower) for seedlings grown in the Catskill Mountains in New York State.

average than the other treatments. Most parameters of the light curves were higher in the samples measured in May of 2006 with reduced assimilation and the appearance of treatment effects in the August sampling period. These will be discussed in a later section.

Compensation Point

Soil treatment did not alter the photosynthetic compensation point of seedlings ($F= 0.91$, $P =0.4420$) or mature trees ($F =2.14$, $P =0.1017$). However, this parameter varied significantly by time ($F= 4.82$, $P=0.0113$) (Table 3.3). The compensation points of the mature trees were lower than that of the seedlings in May of 2006.

Dark Respiration

Dark respiration was not significantly affected by soil treatments in seedlings ($F = 1.54$, $P = 0.2982$) or in trees ($F =1.04$, $P = 0.3777$). However, dark respiration varied significantly by time for both seedlings ($F =17.70$, $P < 0.0001$) and trees ($F =3.14$, $P = 0.0491$) (Table 3.3).

Table 3.3. Descriptive parameters derived from light response curves for sugar maple a) mature trees and b) seedlings growing in plots exposed to supplemental nitrogen and calcium treatments in New York State's Catskill Mountains. Each value is the mean of the three measurement periods and is expressed in μmol m^{-2} s^{-1}.

a.

Parameter	Treatment	Mean	Error	F-statistic	P-value
Dark Respiration	Control	-0.4505	0.8318	4.26	0.0763
	Ca	-4.3050	0.8318		
	N	-1.4550	0.8318		
	Ca + N	-1.1497	0.6791		
Compensation Point	Control	32.5	12.4	9.24	0.587
	Ca	7.3	20		
	N	12.3	21.4		
	Ca + N	56	35.2		
AQE	Control	0.016	0.0061	0.67	0.5764
	Ca	0.023	0.0040		
	N	0.016	0.0075		
	Ca + N	0.014	0.0075		
An at Saturation	Control	2.0990	0.2926	2.40	0.0727
	Ca	1.3085	0.2776		
	N	1.4562	0.3318		
	Ca + N	2.2374	0.3104		

b.

Parameter	Treatment	Mean	Error	F-statistic	P-value
Compensation Point	Control	34.22	8.48	0.34	0.7958
	Ca	28.63	8.30		
	N	41.93	10.50		
	Ca + N	35.84	9.09		
Dark Respiration	Control	-1.1212	0.8318	4.26	0.0984
	Ca	-0.7596	0.8221		
	N	-1.2646	0.8318		
	Ca + N	-0.7347	0.6791		
An at Saturation	Control	1.9865	0.2045	1.30	0.2765
	Ca	2.1265	0.1829		
	N	1.7342	0.2361		
	Ca + N	2.3383	0.2112		
AQE	Control	0.0175	0.002088	0.73	0.5385
	Ca	0.0194	0.002044		
	N	0.0147	0.002503		
	Ca + N	0.0182	0.002239		

Table 3.4. Main effects of soil treatments on dark respiration for a) mature and b) sugar maple seedlings growing in plots of supplemental Ca and N in the Catskill Mountains, New York State. * denotes $P < 0.05$, ** denotes $P < 0.0001$.

a.

Source	DF	Type III SS	MSE	F Value	P Value
Block	2	2.4060	1.2030	0.69	0.5063
Treatment	3	5.4895	1.8298	1.04	0.3777
Block*Treatment	6	3.2267	0.5378	0.31	0.9315
Time	2	10.9851	5.4926	3.14	0.0491 *
Treatment*Time	6	6.0716	1.0119	0.58	0.7470

b.

Source	DF	Type III SS	MSE	F Value	P Value
Block	2	2.01119	1.00559	1.83	0.1664
Treatment	3	3.06105	1.02035	1.86	0.1427
Block*Treatment	6	3.97553	0.66258	1.21	0.3107
Time	2	19.43779	9.71888	17.70	<0.0001 **
Treatment*Time	6	8.335501	1.38925	2.53	0.0264 *

Assimilation at Saturation

The rate of CO_2 assimilation at saturating light levels on mature trees or seedlings was not affected by soil treatments (Table 3.3). However, there was a block effect and a block by treatment interaction in the seedling response (Table 3.5). This may have been due to variations in the response of the soils or update by the plants as noted previously for the variability in these parameters. This value also changed over time, suggesting, as with light compensation and AQE, that the seedlings altered their photosynthetic physiology as the season progressed. Assimilation was the highest in June, perhaps when water was most abundant. Seedlings growing on plots with the smallest inclination (e.g. flatter plots) also had higher light-saturated assimilation rates and this may also be indicative of moister more poorly drained soils.

Table 3.5. Main effects of soil treatments on light-saturated CO_2 assimilation for a) mature and b) sugar maple seedlings growing in plots of supplemental Ca and N in the Catskill Mountains, New York State. * denotes P < 0.05, ** denotes P < 0.0001.

a.

Source	DF	Type III SS	MSE	F Value	P Value
Block	2	5.57	2.79	1.12	0.3313
Treatment	3	15.79	5.26	2.12	0.1046
Block*Treatment	6	6.95	1.16	0.47	0.8320
Time	2	5.70	2.85	1.15	0.3231
Treatment*Time	6	5.15	0.86	0.35	0.9109

b.

Source	DF	Type III SS	MSE	F Value	P Value
Block	2	5.3671	2.6835	3.13	0.0479 *
Treatment	3	4.7597	1.5866	1.85	0.1428
Block*Treatment	6	19.5761	3.2627	3.80	0.0018 *
Time	2	44.5976	22.2988	25.98	<0.0001 **
Treatment*Time	6	3.2110	0.5352	0.62	0.7110

Apparent Quantum Efficiency

Soil treatments had no effects on AQE for either seedlings (F =1.56, P= 0.2082) or mature trees (F =1.36, P=0.2730). However, significant differences were, again, seen across levels of time, with the highest AQE observed in sugar maples seedlings in May of 2006 (F =4.87, P= 0.0108).

Table 3.6. Main effects of soil treatments on apparent quantum efficiency (AQE) for a) mature and b) sugar maple seedlings growing in plots of supplemental Ca and N in the Catskill Mountains, New York State. * denotes $P < 0.05$, ** denotes $P < 0.0001$.

a.

Source	DF	Type III SS	MSE	F Value	P Value
Block	2	0.0001948	0.0001948	0.54	0.4678
Treatment	3	0.001470	0.0004902	1.36	0.2730
Block*Treatment	6	0.0007351	0.0002450	0.68	0.5712
Time	2	0.0001581	0.0001581	0.44	0.5128
Treatment*Time	6	0.00011923	0.0001192	0.33	0.5694

b.

Source	DF	Type III SS	MSE	F Value	P Value
Block	2	0.0001425	0.00007129	0.81	0.4494
Treatment	3	0.0004115	0.0001372	1.56	0.2082
Block*Treatment	6	0.0004075	0.0006792	0.77	0.5950
Time	2	0.0008568	0.0004284	4.87	0.0108 *
Treatment*Time	6	0.0003540	0.0005900	0.67	0.6738

Microcalorimetry

R_q

R_q, the measurement of heat produced by respiring tissues, is a measure of anabolism and catabolism per milligram of dried tissue. No significant differences were seen in R_q in mature tree foliage or seedlings in response to soil treatment (Table 3.7). However there were significant block and time effects for mature trees and time effects for seedlings (Table 3.8). Foliage sampled in August of 2005 had significantly lower R_q than at other sampling times ($F=289.87$, $P <0.0001$) (Table 3.8). Analysis of the simple means alone also revealed no statistically significant effects of soil treatments.

Table 3.7. Metabolic measurements from microcalorimetric methods for a) mature trees and b) seedlings of sugar maples grown in plots subjected to 4 different treatments of nutrient additions in the Catskill Mountains, New York State. Each value is the mean and standard error of 4 subsamples from each plot. All values are given in microwatts. * Denotes $P < 0.05$, ** denotes $P < 0.001$.

a.

Treatment	Rq	Rco2	Rsg	Rq/Rco2
Control	3803 (632)	6.07 (1.74)	-331.63 (604)	731.9 (175)
Ca	4355 (579)	8.79 (1.94)	-59.81 (618)	530.6 (179)
N	4071 (624)	8.47 (1.94)	718.99 (666)	488.7 (195)
Ca + N	4062 (632)	7.19 (1.89)	-954.47 (657)	576.6 (193)

b.

Treatment	Rq	Rco2	Rsg	Rq/Rco2
Control	2917 (1257)	6.31 (2.90)	49.98 (512)	855.3 (427)
Ca	2417 (1200)	3.45 (2.36)	-792.63 (510)	15.72 (422)
N	1483 (907)	2.28 (1.98)	-413.43 (627)	927.33 (523)
Ca + N	2978 (854)	3.28 (2.33)	-518.32 (561)	1145 (468)

Table 3.8. ANOVA tables for Rq for a) mature and b) seedling sugar maples in response to 4 soil amendment treatments in New York's Catskill Mountains. Results are given in microwatts and are the means and standard error of 3 subsamples from 4 treatment plots in 3 blocks. * Denotes $P < 0.05$, ** denotes $P < 0.001$.

a.

Source	DF	Type III SS	MSE	F Value	P Value
Block	2	3.0 x 107	1.5 x 107	4.54	0.0123*
Treatment	3	1.9 x 106	4.4 x 106	4.54	0.2767
Block*Treatment	6	2.4 x 106	4.0 x 106	1.19	0.3167
Time	4	1.7 x 108	4.3 x 107	127.23	<.0001 **
Treatment*Time	12	4.5 x 106	3.8 x 106	1.13	0.3440

b.

Source	DF	Type III SS	MSE	F Value	P Value
Block	2	5.9×10^6	3.0×10^6	0.67	0.5442
Treatment	3	1.2×10^7	4.1×10^6	0.92	0.4842
Block*Treatment	6	1.8×10^7	3.0×10^6	0.68	0.6734
Time	4	1.3×10^8	1.3×10^9	289.87	<0.0001 **
Treatment*Time	12	1.2×10^6	4.0×10^6	0.92	0.4549

Rco2

As for R_q, there was no significant Rco2 response to soil treatments in mature tree or seedling leaf tissue (Table 3.7). However, again there were significant block and time effects for mature trees and time effects for seedlings. Rco2 measurements from June 2005 and May 2006 showed a higher average value than measurements taken throughout the rest of the year, and mature trees on level ground had higher Rco2 data points in general. Seedlings measured in 2005 showed higher levels of Rco2 than seedlings in 2004 ($F = 22.01$, $P = 0.0034$) (Table 3.9).

Table 3.9. ANOVA tables for Rco2 for a) mature and b) seedling sugar maples in response to fertilizer treatments in New York's Catskill Mountains. Results are given in microwatts and are the means and standard error of 3 subsamples from 4 treatment plots in 3 blocks. * denotes P < 0.05, ** denotes P < 0.001.

a.

Source	DF	Type III SS	MSE	F Value	P Value
Block	2	1111.60	555.80	7.20	0.0011 *
Treatment	3	248.94	82.98	1.08	0.3618
Block*Treatment	6	265.65	44.27	0.57	0.7508
Time	4	8880.17	2220.04	28.77	<0.0001 **
Treatment*Time	12	870.83	72.57	0.94	0.5091

b.

Source	DF	Type III SS	MSE	F Value	P Value
Block	2	7.35	3.68	0.26	0.7789
Treatment	3	34.95	11.65	0.83	0.5249
Block*Treatment	6	73.91	12.32	0.88	0.5620
Time	1	309.58	309.58	22.01	0.0034 *
Treatment*Time	3	34.96	11.65	0.83	0.5248

Rsg

The amount of energy available for growth in mature tree or seedling foliage (Rsg) showed results parallel to that of the other parameters and showed no significant response to soil amendments (Table 3.10). However, there were significant differences detected for the block effect. (F =7.98, P =0.0005) and across sampling times (F= 4.26, P= 0.0028).

Table 3.10. ANOVA tables for Rsg for a) mature and b) seedling sugar maples in response to fertilizer treatments in New York's Catskill Mountains. Results are given in microwatts and are the means and standard error of 3 subsamples from 4 treatment plots in 3 blocks. * denotes $P < 0.05$, ** denotes $P < 0.001$.

a.

Source	DF	Type III SS	MSE	F Value	P Value
Block	2	2.3×10^9	1.2×10^8	7.98	0.0005 *
Treatment	3	1.9×10^8	6.5×10^6	0.45	0.7196
Block*Treatment	6	1.6×10^8	2.7×10^7	0.19	0.9793
Time	4	2.4×10^9	6.2×10^7	4.26	0.0028 *
Treatment*Time	12	1.4×10^9	1.2×10^7	0.80	0.6487

b.

Source	DF	Type III SS	MSE	F Value	P Value
Block	2	2.9×10^6	1.4×10^6	0.77	0.5095
Treatment	3	2.7×10^6	9.2×10^5	0.50	0.7007
Block*Treatment	6	5.1×10^6	8.5×10^5	0.47	0.8107
Time	1	4.8×10^6	4.9×10^6	2.63	0.1659
Treatment*Time	3	2.7×10^6	9.2×10^5	0.50	0.7008

Rq/Rco2

Rq/Rco2, or metabolic efficiency, was not affected by soil treatments in either mature trees or seedlings (Table 3.7), although in the mature trees, Rq/Rco2 was significantly affected by block with the lowest values found on plots with the least slope ($F = 3.55$, $P = 0.0313$) and in June 2005 ($F = 4.74$, $P = 0.0013$).

Table 3.10. ANOVA tables for the ratio of Rq/Rco2 for a) mature and b) seedling sugar maples in response to fertilizer treatments in New York's Catskill Mountains. Results are given in microwatts and are the means and standard error of 3 subsamples from 4 treatment plots in 3 blocks. * denotes $P < 0.05$, ** denotes $P < 0.001$.

a.

Source	DF	Type III SS	MSE	F Value	P Value
Block	2	0.0001948	0.0001948	0.54	0.4678
Treatment	3	0.001470	0.0004902	1.36	0.2730
Block*Treatment	6	0.0007351	0.0002450	0.68	0.5712
Time	2	0.0001581	0.0001581	0.44	0.5128
Treatment*Time	6	0.00011923	0.0001192	0.33	0.5694

b.

Source	DF	Type III SS	MSE	F Value	P Value
Block	2	0.0001425	0.00007129	0.81	0.4494
Treatment	3	0.0004115	0.0001372	1.56	0.2082
Block*Treatment	6	0.0004075	0.0006792	0.77	0.5950
Time	2	0.0008568	0.0004284	4.87	0.0108 *
Treatment*Time	6	0.0003540	0.0005900	0.67	0.6738

Chapter 4: Discussion

Soil Treatments and Foliar Responses

The treatment of ammonium nitrate and dolomitic limestone proved to be effective in amending the soil in these plots, as has been shown by other fertilization studies (Gundersen 1991, Magill et al., 1997). Soil from calcium treated plots had nearly three times the soil calcium levels found in untreated plots. Nitrogen addition led to doubled soil nitrogen concentrations as compared to control plots. However, it was unexpected to see lower nitrogen levels in soil from calcium treated plots and calcium/nitrogen treated plots. It is not clear whether this was the result of Ca addition, perhaps by increased N uptake by plants in those plots, or by some other factor.

Calcium additions also raised soil pH making these soils a full magnitude more alkalkine than control plots. Calcium addition, whether added with nitrogen or alone, led to trends of increased CEC although the significance level was only 0.06. It is possible that these factors also contributed to higher biomass and/or plant uptake of N on these plots. Measurements of plant productivity on these plots would have allowed to evaluate total N in the plots instead of simply N concentrations.

Aluminum levels were also significantly higher in both calcium and calcium/nitrogen plots. Magnesium levels, while not significant, were found in lower concentrations for both calcium and calcium/nitrogen plots than control plots. Overall, calcium addition and calcium/nitrogen addition had very similar effects on soil chemistry.

The addition of nitrogen also altered the soil chemistry of the plots. Nitrogen fertilization did not alter soil pH or aluminum concentrations but Nitrogen treated plots did, however, show minor drops in magnesium concentrations and cation exchange capacities, but these were not statistically significant. These measurements indicate that even with double the ambient nitrogen deposition treatments, these soils changed very little. Analysis of water chemistry from streamflow in this watershed would help in understanding if these plots are truly nitrogen saturated in following with studies done with Vitochek (1997, 2000) and Driscoll (2001), this soil analysis suggests that these soils are truly nitrogen saturated.

In general the nutrient additions used in this study produced soil chemistry results that for the most part echoed results from previous studies. The addition of calcium and nitrogen to forest soils were effective in altering relative pH, calcium, and aluminum concentrations as seen in other soil amendment studies (Houle et al., 2002, Guyette et al., 1992, Juice 2006, Wargo et al., 2000). However, in this study soil nitrogen concentrations decreased with the addition of calcium, which seemed to be the opposite response from what was seen in both Wargo's (2002) and Juice's (2006) calcium amendment studies. The difference between our results and Juice and Wargo's studies may be due to one of two factors: 1) difference in study design, or 2) calcium addition promotes excessive nitrogen uptake in either sugar maples or other species. First, both Wargo and Juice used repeated measures of soil concentrations throughout the year, while in this study soil samples were taken only once in May of 2007. Certainly a more statistically viable design would include a multiple samples to account for soil chemistry differences due to weather or season, since both alter

soil nitrogen dramatically (Aber 2000). Sampling techniques in this study were limited by both time and funding, leaving samples to be taken only once. Second, it is possible that soil N, as hypothesized, was depleted in calcium treated plots and the addition of this nutrient at the surface level immediately promoted the growth of herbivorous ground cover, especially the abundant Wood Fern (*Dryopteris intermedia)*. With calcium no longer a limiting factor in the upper horizons where Wood Fern's root mass lies, nitrogen is reallocated into the tissues of this groundcover while being depleted in the soil solution.

Observations of the plots bolster this hypothesis. The control plots or plots with calcium addition alone had a wide variety of fern, spring ephemeral and perennial species represented on the forest floor. However the three nitrogen and the three nitrogen/calcium treated plots showed a thick, nearly impenetrable thicket of common wood fern in a monocultural blanket (Fig. 4.1b).

Ferns in such plentitude may not have any effect on mature trees, but for sugar maple seedlings this dense ground cover can lead to shading so severe that many of the sugar maple seedlings thriving in early summer were found dead from overcrowding by August. While sugar maple is a shade obligate species at the age of seedlings, the cover was so dense that no light could penetrate to the seedlings at all (St Clair 2004). While there has been little investigation in Eastern forests and the interaction of soil nitrogen and fern species, Fenn (1996) found that bracken fern in California's San Bernadino Mountain is so closely linked with high levels of nitrogen that its overwhelming presence on the forest floor is used as a nitrogen excess indicator (Fenn 1996, Fenn 2000). Fenn's studies have also found that ferns take up a

Illus. 4.1. Control plots (a) had an open herbaceous layer as compared to nitrogen addition plots (b), where a dense monoculture of Common Wood Fern *Dryopteris intermedia* shades out other herb species and seedlings common in along the Catskill forest floor.

a.

b.

large proportion of nitrate in the soil (2000). This was especially seen in these plots, where plots receiving both calcium and nitrogen showed soil chemistry almost identical to plots receiving calcium alone. Since calcium is an essential nutrient for ferns as well, the lack of such fern density is understandable in plots receiving only nitrogen. Gill and Marks (1991) have also seen strong positive correlations between thick herbaceous layers and the increased rates of seed predation, presumably since the presence of a protective layer of fern allows rodents and birds to scavenge more thoroughly, which helps explain the lack of seedlings and saplings in these plots.

Foliage from nitrogen amendment plots with or without calcium showed nitrogen concentrations that tended to be higher than control foliage but only approaching levels of significance at P=0.07 and 0.10, respectively. Calcium treatment, however, led to foliage with significantly higher levels of nitrogen than foliage grown in untreated plots. Calcium treatment has resulted in increased nitrogen content in other studies (Juice et al., 2006), though the resulting foliar nitrogen was much larger than this study. Juice's study also covered two years longer after fertilization applications than this study, so it is possible that had this study continued then higher foliar nitrogen would be seen here as well.

Since the addition of N did not increase foliar N levels this may further support the N saturation hypothesis or to the enhanced availability and uptake of N by competing species. This is consistent with other sugar maple liming studies (Wargo et al., 1997) and nitrogen addition studies (Carmean and Watt 1975). They suggested that sugar maple does not respond to nitrogen, phosphorus, or potassium inputs when foliar nitrogen is greater than 2% as was seen in this study, so these results are

consistent with those studies. Cote et al. (1993), using a range of seven different fertilizers, evaluated the range of foliar concentrations for "healthy" sugar maples in Quebec and found that the critical level of nitrogen was 2000 mg/kg^{-1}. This indicates that the trees and seedlings evaluated in this study were not in critically deficient condition. Kolb and McCormick published even broader ranges of acceptable foliar concentrations for sugar maple in 1993, and when compared to the results of this study, suggest that the sugar maples in this study were definitely nitrogen abundant. Unfortunately, the foliar concentrations of other chemicals, most especially calcium, were not evaluated in this study but have been extremely useful in assessing the overall impacts of the soil amendments.

Physiological Responses

Since it has been determined that up to 30% of total leaf's nitrogen content is within the structure of Rubisco (Evans 1989), and because is was assumed that carbon fixation activity is in proportion to the concentration of nitrogen within the leaf (Amthor 1984, Friend 1989), it would be expected that the rates of carbon assimilation would be increased with the addition of nitrogen. Also if Ca deficiency was present and limiting plant vigor it would likewise be expected that that Ca additions would also enhance photosynthesis. However, neither gas exchange nor metabolic measurements were altered by the soil treatments. The small and in most cases statistically insignificant increases in foliar N levels may not have been enough to increases photosynthetic or total metabolic activity, which may have been already limited by other environmental factors. Also shade trees, such as sugar maple, have

much lower percentages of nitrogen in their foliage devoted to photosynthetic elements such as Rubisco (Chazdon and Field 1987) so this may also have dampened any response in photosynthesis. Calcium addition had a trend toward reducing dark respiration and this could in itself increase net photosynthesis but no clear trends could be detected in this study where the rates were quite variable. Assessments of plant growth or productivity in the plots may have given a more integrated approach to the question of reduced stress or enhanced productivity in these plots.

Other studies utilizing soil amendments have shown inconsistent responses in terms of changes in plant gas exchange. For example St. Clair (2005) found no correlation between nitrogen additions and photosynthetic efficiencies, a finding echoed here. Other nitrogen addition studies have also been conducted on sugar maple and eastern hardwood forests, but these studies were conducted on nitrogen-poor soils, not the nitrogen saturated soils seen in the Catskills (Ellsworth 1998, Binkley and Reid 1984). Nevertheless, both Ellsworth (1998) and Binkley and Reid (1984) found that sugar maple responded to nitrogen additions with increased crown density, reduced dieback and improved foliar nutrition. Previous calcium addition studies (Juice 2006) have shown increases in vigor as determined from measurements of fluorescence and class ratings in canopy transparency, dieback and vigor, but we have not found in other instances where gas exchange measurements have been conducted on calcium treated sugar maples in the field.

Considering past studies, the lack of response to nitrogen addition is not surprising. While some nitrogen fertilization studies have shown increases in growth in sugar maple, there have also been studies with no response (Gilliam et al., 1996,

Magill et al., 1997, Aber et al., 1998). The lack of responses could be due to N saturation. The response of sugar maple to nitrogen when nitrogen is found in excess is thought to follow the pattern described by Aber and others (1998), where steady rates of nitrogen deposition results in increases in foliar nitrogen. As calcium:aluminum ratios, magnesium:nitrogen ratios and soil acidification increases, sensitive plant species and microbes begin to decline. The microbial population shifts and net primary productivity (NPP) begins to plateau (Aber et al., 1998). This may have been the case in this study. However, it is also possible the limited measurements periods, low replication and gas exchange methodology utilized here was not sufficient to detect small changes in gas exchange or metabolic parameters.

Measurements taken through microcalorimetry, like measurements taken through gas analysis, showed no significant effects and no obvious trends in response to soil treatment in any of the five parameters measured. From these results, soil treatment had no effect on the anabolic or catabolic processes of sugar maple tissue, either mature or seedling.

Although there were few effects of soil treatment on photosynthesis and metabolism, the effect of time was significant in all three of the gas analysis parameters measured on seedlings and two of the three measurements on trees (assimilation at saturation showed no time significant time effects). A time effect was seen on metabolic measurements as well, with significant time responses for mature and seedlings for both Rq and Rco2. Sugar maples are very sensitive to abnormal weather events, especially unusual freezing and thawing occurrences (Decker et al., 2003, Groffman et al., 2001, Horsley et al., 2002). These events may lead to damage

that has a very strong negative effect on growth, such as fine root death, failure to create mycorrhizal associations, and fluxes in nitrogen cycles when nutrients are most needed (Tierney et al., 2003). Sugar maples are also extremely sensitive to drought (Allen et al., 1992, Kolb and McCormick 1993, Ni and Pillardy 1993, Dawson 1993). The two years when measurements were taken had very low rainfall in the summer months, most especially early summer when growth rates are at their highest (NOAA). In general, growth and photosynthetic activity was highest in the measurements taken in the early summer of 2005 and 2006 with greatly decreased activity seen in all measurements in late summer 2005. This is reasonable for sugar maple metabolically, as anabolic activity would be at it's highest in times when tissues are young and developing (Hansen and Criddle 1997). This is especially true in sugar maple, a species that has a single flush of leaves for the entire growth season. Photosynthetic response throughout the growth season in non-stressed conditions might be expected to be higher later in the summer when leaves would be fully developed and pigments are at their highest concentrations. This was not seen in this study, perhaps an indication that as drought progresses and more soil nutrients are allocated to biomass, tissues become even more nutrient stressed and at a magnitude even stronger than the treatment response (Nonami and Schulze 1991, Barber 1995).

The block effect of slope and altitude was also found in a number of the calorimetric measurements, and was found to be significant in mature trees and once when measuring carbon dioxide assimilation at saturation in seedlings. Plots were blocked for both altitude and slope in this study, with a maximum of 500 feet in elevation difference and flat versus a slope of approximately 40 degrees at its

steepest. The terrain of the lowest plots in comparison to the highest plots was observed to be obviously different when the harvesting of soil in the upper plots showed mineral soil horizons of no more than six centimeters before reaching parent material or buried boulders. Significant differences in slope effect has been found in previous nutrient-addition sugar maple studies as well, presumably due to increase in runoff and leaching (St. Clair 2004, Lovett 2000, Ledig 1983). In following with Lovett (2000) and St. Clair's work (2004), the sugar maples in this study on relatively level ground and at a lower altitude showed a significant increase in photosynthetic and metabolic response. These lower plots were observed to have a dramatic increase in organic horizons, and the water retention was remarkably higher than in the steeper plots. Since this species is so susceptible to drought, the thicker O horizon and level ground may help to retain many of the nutrients and soil solution that are leached on steeper plots (Lovett 2000).

A number of issues in this study may have contributed to the high level of variability in the data. Clearly, a main problem with this study is the small sample size, both of mature leaves and seedlings. Harvesting in general proved to be a problem, since the shooting of foliaged leaves from the canopy resulted in many leaves perforated and torn with buckshot. Many larger leaves were damaged and unusable, and so smaller, younger leaves were used for calorimetry instead, possibly skewing the metabolic means. Seedling harvesting was hindered greatly simply by the lack of seedlings in the plots – the 2006 harvest yielded only 10 seedlings total, making statistical analysis difficult. The collection of more samples from both mature and seedling leaves was also limited by time and manpower since it was

essential to minimize the duration from harvest to metabolic measurements. Criddle and Hansen (2005) have shown that metabolism as assessed by microcalorimetry, is influenced by the tissue age, especially in a species that produces a single flush of leaves a growing season. While it would have been most fascinating to observe the changes in metabolic rate in response to not only treatments but also to age of the tissue, the sample sizes in this study simply did not allow for this.

The metabolic measurements had extremely large errors in all measurements, which may be related to wound response of the tissue due to folding, the build up of toxic products within the leaf, or the effect of biofilms (Hansen and Criddle 1989, Hansen and Criddle 2005, Criddle 1999). Each leaf sample was gently patted down and acclimatized to room temperature before measurements began to eliminate the problem of water droplets evaporating from the leaf surface and modifying heat exchange. There is also the concern of the doubtlessly extremely active metabolism taking place in the diverse communities inhabiting the phyllosphere. Sugar maples, especially compromised individuals, have a thriving biofilm and microscopic insect community living on each leaf's surface (Legace et al., 2006). Many samples were observed with multiple species of mite colonies, all microscopic and nearly impossible to remove (Ms. Ethyl Dutky, University of Maryland Plant Diagnositc Clinic, personal communication). While care was taken to avoid using these visibly afflicted leaves for calorimetric measurements, it is beyond question that many mites, bacteria and other organisms were accidentally included in these measurements. Since microcalorimetry is used to assess biofilms in other studies, the accidental inclusion of the biofilm's respiration may add variation to the results (von Rege and

Sand 1998, Wentzien 1994). Due to the small sampling size and the spatial range of the mite habitat, it is quite possible that the error due to natural variability factors like this one was not eliminated entirely.

While this study did not result in any clear solutions to remedy the decline of sugar maple forests, it did show the intricate ecological complexity of the issue at hand and raise the possibility of a number of environmental players. If just herbivory, fern cover, and drought created such an impact on this single short-term nutrient study, it is baffling to think of the thousands of factors that may impact the ecology of sugar maple stands. For instance, while the primary pathways of nutrient stress in sugar maple have received a great deal of attention recently, secondary issues are rarely focused upon. One such issue is the factor of age. Mohammed's study (1997) investigated the relations of aluminum to sugar maple decline and found that older sugar maple had accumulated so much aluminum in their xylem throughout their lifetime that the levels were approaching lethal limits.

The age of the Catskill forests in itself may be a strong contributor to its decline. After widespread non-specific logging of the region up until the 1950s, these forests were allowed to regenerate their forests (Evers, 1995). Sugar maples do not reach reproductive maturity until they are 30 years old, and will not usually produce samaras after 60 years of age (Botkin 1993). The observed lack of seedlings and saplings may be due to nutrient deficiency as hypothesized, but it may also be due to the larger picture of the transition of a sugar maple dominated forest to one dominated by yellow beech, red oak and fir.

As the investigation of sugar maple continues, it is has become apparent that the only way to find the effects of this species in relation to nutrient imbalances, anthropomorphic deposition, insect herbivory, regeneration, and climate change is through the combination of long-term field studies and greenhouse simulation work. The use of Criddle and Hansen's latest metabolic heat calculations may be too precise to be used in a field experiment such as this one, but it would be invaluable to observe the metabolic changes of seedlings in a greenhouse setting in response to seasonal temperatures and nutrient additions. A controlled factorial field experiment assessing the additions of many micronutrients – calcium, magnesium, phosphorus, and potassium – and their movements through the soil profile when both nitrate and ammonium are added in differing amounts, would also paint a clearer picture and give essential perspectives of the soil imbalances in sugar maple forests. Whatever aspects this field turns to investigate next, this study has demonstrated that nitrogen and calcium nutrition play a vital role in sugar maple vitality. The addition of calcium to the soil solution resulted in some positive photosynthetic activity and an increase in foliar nitrogen. The addition of nitrogen suggested that the soils may be nitrogen saturated, and that further studies should be conducted to find the relation of nitrogen concentration to photosynthetic response.

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