SUPPORTING INFORMATION

Title: Restoring Soil Calcium Reverses Forest Decline

Authors: John J. Battles, Timothy J. Fahey, Charles T. Driscoll Jr., Joel. D. Blum, and Chris E. Johnson

METHODS

Research site. On average, Hubbard Brook Experimental Forest (HBEF) receives 1,395 mm (std. dev. = 189 mm) of precipitation per year, part of which is captured in snow pack persisting from December until April. The mean annual temperature is $5.5 \,^{\circ}$ C (std. dev = 0.61 $^{\circ}$ C); daily temperatures average from -8.5°C in January to 18.8°C in July¹. The forest of HBEF is considered representative in vegetation and climate of the northern hardwood forest complex, which extends from the north-central United States into southeastern Canada². The soils of HBEF are moderately well-drained Spodosols (Haplorthods) and Inceptisols (Dystrochrepts) of sandy-loam texture formed from glacial till. There is a high degree of spatial variability in the experimental watersheds with shallower, more acidic soils at higher elevations³. These soils exhibit a pattern common in mountainous forest landscapes throughout the Northeast – there are low rates of calcium (Ca) supplied by weathering and the effects of acidic deposition intensify with increasing elevation³.

Forest inventory. The entire reference watershed (WS6) is divided into 208, 25 by 25 m square plots (0.0625 ha, slope corrected). At every five-year interval since 1992, we measure all standing trees ≥ 10 cm dbh in the plot, identify the species, and determine a vigor class based on the health of live trees and the decay class of standing dead trees. Small trees (2.0 to 9.9 cm dbh) are sampled in a 3-m strip along the southern edge of each plot (approximately 75 m²).

Prior to 1992, tree sampling regimes in WS6 varied. In 1965, all trees ≥ 2 cm dbh were measured in a 100 m² square subplot in each of the 208 plots. In 1977, the tree inventory was conducted (i.e., all trees ≥ 10 cm in the 208 plots were measured) but no small trees were measured. In 1982 and 1987, the tree inventory was completed but small trees were measured on random subsets of the 0.0625 ha plots (32 plots in 1982, 35 plots in 1987). These data provide the core information needed to document biomass increment trends and quantify forest productivity responses to the Ca amendment.

Data analysis. As noted, the biomass of living trees was calculated from site-specific allometric equations in which parabolic volume of the tree bole is used to estimate biomass⁴. Given the dependence of biomass on accurate estimates of tree diameter and tree height⁵, we revised our inventory protocols to reduce measurement errors in dbh. We also refit our dbh-to-height equations based on more than 1,300 samples (stratified by species and elevation) collected across WS6 and WS1 in 1996 and 1997 (i.e., prior to the application of Ca). Our approach to estimating biomass at the HBEF has been repeatedly refined and validated against actual measured biomass⁵⁻⁷. On average, there was less than 5% difference between harvest-based and allometric-based estimates of aboveground tree biomass^{6.7}.

We used Monte Carlo randomizations to propagate and combine the errors that contribute to the uncertainty associated with our measurements of tree biomass. The primary sources of error include: 1) measurement error of dbh; 2) variation in the saturating equations predicting tree height from tree diameter; 3) variation in the power equations predicting tree biomass from parabolic volume; and 4) spatial variation among sample plots. It is important to note that at HBEF some populations have been completely inventoried at the watershed scale (e.g., trees \geq 10 cm dbh). In these instances, there is no spatial variation even though we still use plots as a convenient accounting tool. In our forest biomass estimates, we followed the best practices for Monte Carlo propagation of error as recommended in a recent review⁸. We report means and 95% confidence intervals calculated from 1,000 Monte Carlo simulations.

Leaf area index (LAI) was estimated by counting leaves in traps and using measurements of average area per leaf for each species and site. Area per leaf was measured on both reference and treated sites in four years; no significant differences (one-way ANOVA) were observed across years within sites. A correction to hardwood LAI was needed to account for conifer LAI (not sorted from litter traps). This correction was based on allometric estimates of conifer (red spruce, balsam fir) leaf area calculated from the complete survey of trees on the two watersheds described earlier. We also compared watershed-wide estimates of total LAI between the litterfall and allometric approaches, prior to treatment (1997 surveys). These comparisons indicated close agreement between methods (<4% difference). Allometric comparisons also indicated pre-treatment LAI was very similar between watersheds prior to treatment (1% difference). The LAI values in these sites are similar to those reported for other sugar maple dominated forests^{9,10}.

Aboveground net primary productivity (ANPP) was calculated as the annual increase in the biomass of perennial woody tissue plus production of ephemeral tissues (e.g., foliage, fruits)^{4,7}. We used the demographic data to correct for wood production of dying trees¹¹ and ingrowth. Note that for WS1 from 2001 to 2006, we lacked comprehensive demographic data. Thus we used the observed mortality and ingrowth rates from 2006 to 2011 to correct the contribution of wood production to ANPP. This approach assumes demographic rates between 2001 and 2006 were the same as the observed rates for 2006-2001. Three lines of evidence support this assumption. A cohort of trees in WS1 (> 800 individuals) tracked since 1998 showed no difference in annual mortality rate for the two intervals. Furthermore there was no

change in the relative density of standing dead trees in WS1 from 2006 to 2011. In both instances standing dead trees accounted for 12% of the population. Finally, there were only minor fluctuations (<3% change) in live tree density between 2006 and 2011. The annual production of ephemeral tissues (leaf, twig, bud, and fruit) was estimated from the litterfall collections, described above.

Sugar maple response. The dynamics of the vegetation during the course of this study (1996-2012) has been particularly complex as evidenced by the fluctuations in biomass increment (Figure 1A) and productivity (Table 1) of the reference watershed (i.e., WS6). The 1998 ice storm that occurred a year before the study contributed to the volatility. Here we provide details on the species-specific responses to the Ca amendment and further support for our contention that sugar maple was the most responsive species to the treatment.

For the major tree species (S), we calculated the net change in live tree biomass in the watersheds for each of the three five-year measurement intervals (i). We then normalized these changes to the trends observed in WS6. Specifically:

$$\Delta Biomass_{S\Delta i} = WS1_{S\Delta i} - WS6_{S\Delta i}$$
 [Equation S1]

where S = species and Δi represents the net change in live tree biomass (Mg ha⁻¹) for species S over the measurement interval i. If there are no difference in species-specific changes between WS1 and WS6, $\Delta Biomass = 0$. Values > 0 indicate a greater increment in biomass during the interval in WS1 relative to WS6; Values < 0 indicate a smaller increment in biomass in WS1. We expressed changes in $\Delta Biomass$ through time as cumulative function with the initial inventory (1996/1997) set to 0. The overall increase in live tree biomass observed in WS1 (Fig. 1A) was largely driven by a corresponding increase in sugar maple biomass (Figure S2). Differences between the watersheds for most of the other major species were minor. The exception was beech. Its biomass declined on WS1 relative to WS6 (Figure S2). Beech is not considered to be as sensitive to soil Ca availability¹² and thus the absence of a positive response to the Ca amendment on WS1 is not surprising. While the reason for the relative decline of beech in WS1 is unknown, we suspect that competition with a vigorous sugar maple population on WS1 limited its growth and recruitment relative to WS6.

The observed increase in leaf area in WS1 (Figure 1B) can also be attributed to an increase in sugar maple leaf area (Figure S3). For each year we calculated the relative difference in sugar maple leaf area as:

$$\Delta LAI_i = WS1_i - WS6_i$$
 [Equation S2]

where i represents the year, WS1_i is the sugar maple leaf area index for WS1 in year i, and WS6_i is the sugar maple leaf area index for WS6 in year i. Sugar maple leaf area was greater in WS1 for every year measured (Figure S3). The differences peaked in 2008 and remained more than 0.9 m²m⁻² higher through 2010.

We also compared the relative growth rate of sugar maple trees (dbh \ge 10 cm) for the most recent five-year interval (2006/2007 to 2011/2012). Relative growth rate was calculated as:

$$RGR_{k} = \frac{\ln dbh_{k,j+1}}{t} = \ln dbh_{k,j}$$
[Equation S3]

where *k* refers to the individual tree, *j* refers to the census, and *t* refers to the time interval between *j* and $j+1^{13}$. We used hierarchical Bayesian analysis to account for error propagation in tree demographic parameters¹⁴. We report the median values and the 95% credibility intervals. For the entire watershed, sugar maple trees in WS1 grew more than 40% faster than trees in WS6: 1.57% yr⁻¹ (95%CI: 1.52 - 1.62 % yr⁻¹) versus 1.10% yr⁻¹ (95%CI: 1.03 - 1.16 % yr⁻¹). There was also a strong elevation gradient with differences in relative growth rate greater at the higher elevations (Figure S4).

Indicators of acidification stress. Several metrics and limits have been suggested for the protection of forest ecosystems from stress associated with elevated acidic deposition¹⁵. including foliar Ca (> 0.55%)¹⁶, soil % base saturation (> 10-15%)¹⁷ and the molar ratio of calcium to aluminum in the soil solution $(Ca/Al > 1)^{17}$. While these metrics and associated thresholds have been widely used to determine critical loads of acidity, they have rarely been evaluated in the context of field observations demonstrating recovery of impacted ecosystems. To help evaluate the applicability of metrics for forest acidification stress, we calculated their values prior to and following the WS1 Ca addition experiment. Foliar Ca concentrations in sugar maple and yellow birch increased significantly following the treatment¹⁸. Prior to treatment, foliar Ca of sugar maple was below the limit (0.55%), indicative of canopy decline in this species¹⁶ over much of the watershed. After treatment, values exceeded this threshold throughout the watershed. Application of soil % base saturation and soil solution Ca/Al as metrics is complicated by spatial heterogeneity in soil chemistry. Both the soil acidity (%BS) and soil solution (Ca/Ali) vary with depth as does the root density. Nevertheless our measurements support the use of these indicators as a measure of sugar maple health in WS1. Prior to treatment, the overall base saturation of the mineral soil was approximately 9.6%. Not

surprisingly much higher values were observed in the surface organic horizons of the forest floor (Oie 48.7%, Oa 32.9%) and the upper mineral soil (0-10 cm, 12.1%). After Ca addition, forest floor % base saturation increased markedly (Oie 78.6%, Oa 56.0%), while upper mineral soil did not increase significantly (14.3%)²⁰. Molar ratios of Ca to inorganic monomeric Al (Ali) in soil solution may be a better indicator of acidification stress than Ca to total Al ratio, as Ali is thought to be the toxic fraction^{18,21}. Our observations of Ca/Ali show that values in the Oa horizons increased from 1.6 pre-treatment to 6.4 post-treatment, while mineral soil solutions increased from 0.97 to 3.8²⁰. In high elevation (~730 m) deciduous stands where sugar maple response was most apparent, the mean Ca:Ali molar ratio was 1.2 in Oa horizon solutions, decreasing to 0.59 in Bh horizon solutions and 0.39 in Bs horizon solutions²² prior to treatment, and increased to 25, 3.5 and 1.5, respectively after Ca addition.

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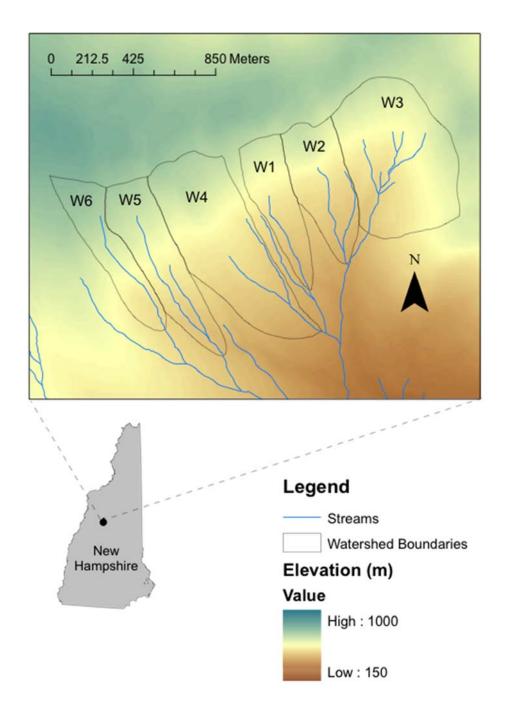


Figure S1. Reference map of watersheds 1 through 6 at the Hubbard Brook Experimental Forest.

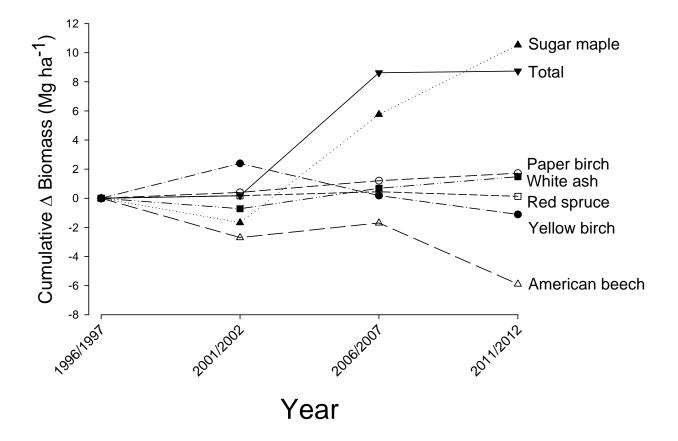


Figure S2. Cumulative change in tree biomass by species during the last 15 years at HBEF. Temporal trends in WS6 were calculated as the baseline (0-line). Positive values represent increases in WS1 relative to WS6. Negative values represent decreases in WS1 relative to WS6.

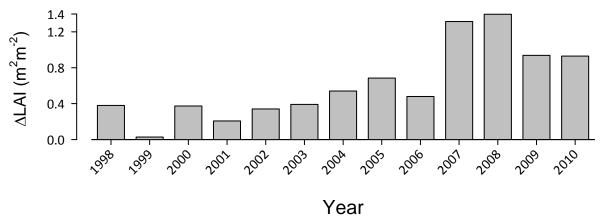


Figure S3. Annual differences in sugar maple leaf area index. Positive values represent

increases in WS1 relative to WS6. Negative values represent decreases in WS1 relative

to WS6.

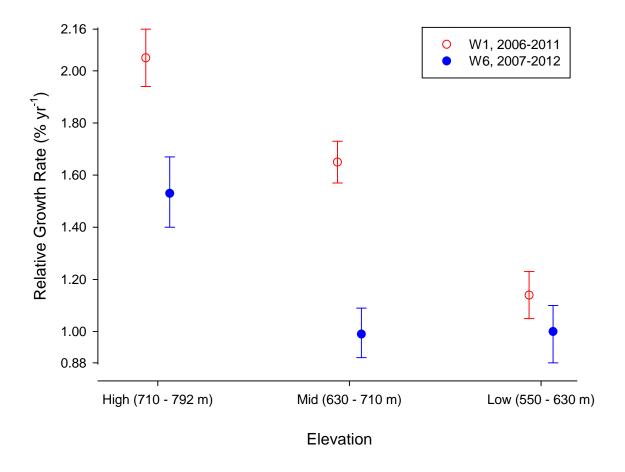


Figure S4. Relative growth rate of sugar maple by elevation band at HBEF. Values represent median growth rates during the most recent five-year interval. Error bars are 95% credibility intervals.

Table S1. Forest composition in the experimental watesheds at HBEF at the start of the experiment (1996 for WS1; 1997 for WS6). Only trees ≥ 10 cm dbh included. Means (with standard errors in parentheses) are reported.

	WS6	WS1
Density (trees ha ⁻¹)	537 (15)	568 (16)
Basal area (m ² ha ⁻¹)	26.0 (0.4)	26.6 (0.4)
Relative Dominance		
(% Basal area)		
Sugar maple	34.2	35.0
American beech	32.2	26.0
Yellow birch	19.9	19.1
White ash	1.2	6.4
Paper birch	6.6	4.2
Red spruce	2.5	4.5
Balsam fir	2.4	1.4