The Effects of Whole-Watershed Calcium Addition on Preferential Uptake of Calcium vs. Strontium and the Isotopic Ratio of $^{87}Sr/^{86}Sr$ into Foliage at the Hubbard Brook Experimental Forest, New Hampshire.

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Introduction

Calcium is an essential if not normally a limiting base cation nutrient in forest ecosystems. The increase in acid deposition (acid rain) in modern times has caused concern about long term leaching of the base cation exchange pool in soils, potentially causing serious damage across northern forests (Likens et al. 1996). Strontium, also an alkaline earth element, is present in trace amounts in rocks and soils. It is thought to be incorporated into plant tissues similarly to calcium because of its ionic size and charge (Ash-Dasch et al. 2006). Strontium forms four stable isotopes in nature ^{84}Sr, ^{86}Sr, ^{87}Sr, and ^{88}Sr (webelements.com). Of these isotopes of strontium, only ^{87}Sr varies due to radioactive decay of naturally occurring ^{87}Rb (Ash-Dasch et al. 2006). Because of this decay different terrestrial geochemical reservoirs develop unique ratios of ^{87}Sr/^{86}Sr over time.

Because calcium and strontium are assumed to have similar geochemical behaviors, the ratio of calcium to strontium (Ca/Sr) and the ratio between the isotopes of ^{87}Sr and ^{86}Sr has commonly been used to trace the sources of calcium in forest ecosystems (Ash-Dasch 2006; Blum et a. 2000). Previous work in this field has established that the uptake rates of Ca and Sr vary between plant species (Ash-Dasch et al. 2006). Correctly interpreting Ca/Sr and ^{87}Sr/^{86}Sr data in order to trace calcium requires understanding the extent to which species discriminate between calcium and strontium during uptake. The discrimination factor (DF) between Ca and Sr can be estimated and applied to nutrient flow studies of calcium through different ecosystems.

This study examines ten years of chemical data from several tree species in Ca-depleted forested watershed. Data came from a whole watershed calcium addition experiment in the Hubbard Brook Experimental-Forest, NH (Peters et al. 2004). The experiment was begun in October of 1999 and sample collection is ongoing. Results for data from 1999-2004 were published in Ash-Dash et al. 2006. Here, I will be updating the data set and calculating discrimination factors after including new data from 2004 through 2008. Following our calculations, a DF greater than 1 indicates a plant's preference for calcium over strontium, and a DF less than one indicates a preference for strontium over calcium.

Widespread calcium depletion has been documented across northeastern North America, including the watershed studied here. The depletion is due to increased acid deposition (Likens et a. 1998). The consequences of calcium depletion on northern forests are only partly understood. At the Hubbard Brook Experimental Forest unexpected declines in biomass accumulation have been observed since the late 1980's, which may relate to increased acid deposition and subsequent leaching of calcium from the nutrient pool (Likens et al. 1996). While some research (focused on global sulfur and nitrogen cycles) on acid rain has indicated that the effects of acid rain on forest nutrient cycles would be limited, there is evidence that the effects of changes in calcium cation pools on forest health could be much stronger(Likens et al. 1996). Spruce (*Picea spp.*) trees are particularly vulnerable to calcium deficiencies in the form of increased freezing injuries in low Ca environments (DeHayes 1999). The vulnerability of forests to calcium depletion and the damage already done to them by acid rain are reasons why being able to trace ecological sources of calcium could aid in long term forest preservation and help inform environmental policy.

Adding calcium to an entire watershed (whole-watershed addition) allows for observations to be made about many aspects of calcium's role in ecosystem health, and how this nutrient cycles through the ecosystem (Peters et a. 2004). The ratio of calcium to trace amounts strontium, and the isotopic ratio of ^{87}Sr to^{86}Sr in the source calcium added to the ecosystem can be used as a chemical marker to follow the added calcium as it flows through the ecosystem. In tracing that marker, observations can be made about relative uptake rates and absorption of calcium and strontium by various plant species. Because of divergent calcium chemistry between plants, characteristic preferences for calcium vs. strontium can then be applied to more reliably trace calcium sources over time in ecosystems.

Site description

The Hubbard Brook Experimental Forest is located in the White Mountain National Forest, New Hampshire. The reserve covers 12 square miles and is managed by the USDA Forest Service Northern Research Station (The National Science Foundation, 2010). The bedrock in the area is mostly Silurian age silimanite schist of the upper Rangeley formation, covered by Pleistocene glacial deposits of varying thickness overlain by spodosol soils up to 60cm thick (Peters et al. 2004).

The ecosystem is a northern hardwood forest with some altitudinal variation of species composition. The understory is predominately viburnum and wood fern. The over story is dominated by mature hardwoods like sugar maple, beech, and birch, with spruce and fir occurring only in the upper reaches of the watershed. The entire area was extensively logged early last century and has since been mostly undisturbed with the exception of a hurricane in 1938 (Ash-Dasch 2006; Likens et al. 1994). The ecosystem study area (Figure 1) is divided into six distinct watersheds. Each watershed can be given various treatments and the natural boundaries of the watershed serve to isolate the effects of the treatment. For this experiment watershed 1 was the subject of a long term calcium addition experiment beginning in October of 1999.

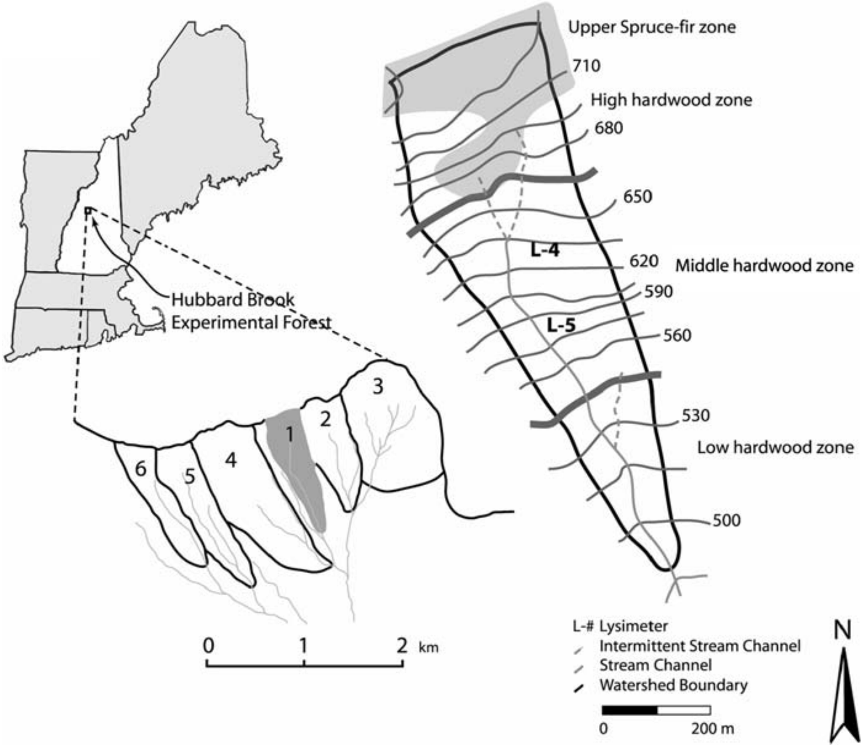
Materials and Methods

The calcium addition was achieved by dispersing fine pellets of amalgamated wollastonite ($CaSi0_3$) powder over the entire watershed by helicopter in the fall of 1999. The wollastonite used in this experiment came from the No. 4 quarry of the Valentine Mine in the Adirondack Mountains of New York (Peters et al. 2004). Ash-Dasch (et al. 2006) found the wollastonite to have a consistent ^{87}Sr /^{86}Sr ratio of 0.70588 (±0.00003, 2σ) and a mean Ca/Sr value of 2870 (±36, 1σ). Ca/Sr ratios of samples collected from the field in years following their addition show no statistical difference from this original value, and indicate that no significant differential dissolution of wollastonite is occurring over time (Ash-Dasch et al. 2006).

Sample Collection

Watershed 1 ranges from 480m to 747m in elevation. The slope was split into four elevation categories, low (480-545m), mid (550-665m), upper (670-700m), and high (>700m) elevations. Seven species of vascular plants common in the area were chosen to sample. These were American beech (*Fagus grandifolia),* balsam fir (*Abies balsamea*), red spruce (*Picea rubens*), sugar maple (*Acer saccharum*), yellow birch (*Betula alleghaniensis),* viburnum (*Viburnum alnifolium*), and wood fern (*Dryopetris spinulosa*) (Ash-Dasch et al 2006). These plants represent the diversity of the ecosystem and come from a wide range of taxonomic families. From an evolutionary and plant systematic perspective, this broadens the range of behaviors we can expect to see in the absorption of calcium. *Spruce* and fir are only found in high elevation zones due to environmental constraints. Composite foliage samples were collected in the watershed each summer from 1999 to 2008. Understory specimens were taken by hand-grabs along four transects in watershed 1, one transect per elevation zone. Transect paths were chosen to cross near lysimeter installations. Overstory samples were taken from the same five to ten individual trees, and collected by shotgun in late summer. Sugar maple is uncommon in 'upper' and 'high' elevation zones.

Figure 1. After Ash-Dash et al. 2006



Data only exists for sugar maple 'upper' from 1999-2001 most likely because sample collectors could not find sugar maple at that elevation. Sugar maple 'high' data only exist for overstory samples. Because of their limited elevation range, spruce and fir needles from the current year were categorized as 'new' and the previous years' needles as 'old'.

Chemical and Isotopic Analysis

Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to analyze the chemical content of the samples for calcium, strontium, and other major and minor cations. Uncertainties are generally ±5% of known values for Ca and Sr based on repeated measurements of laboratory standards. Isotopic data for strontium was obtained using a Finnigan MAT 262 thermal ionization mass spectrometer (TIMS) following the methods used by Ash-Dasch et al. 2006. 2SE uncertainties are reported as ±0.000020 of the $^{87}Sr/^{86}Sr$ ratio. Ash-Dasch et al. (2006) provide a concise description of the exact procedure used to chemically process the samples.

Calculations

Linear regressions were calculated in Microsoft Excel for every species and elevation group using the $^{87}Sr/^{86}Sr$ ratio as on the y axis and the Ca/Sr ratio on the x axis. Data for each species was regressed together (the "All" category in table 3) and separately according to elevation zone. Theoretically Ca/Sr and $^{87}Sr/^{86}Sr$ ratios should begin to trend towards the value of the source wollastonite (2870, 0.70588) after it was added to the system in 1999. In order to investigate variations in Ca vs. Sr uptake between plant species, the data were regressed without including wollastonite value. The linear equation resulting from the regression was used to project the trend in the data to the $^{87}Sr/^{86}Sr$ (y-axis) value of wollastonite to find the projected value of Ca/Sr (x-axis) for the species in question. This projected value was used to calculate a discrimination factor (DFwoll) for the incorporation of strontium relative to calcium in foliage (see eq. 1) using the known Ca/Sr values for wollastonite (as above). A separate DF calculation (DFstream) was also performed using an inferred stream water source (post application) value for Ca/Sr and $^{87}Sr/^{86}Sr$ as an independent check (Ash-Dasch et al. 2006).

Variation in the Ca/Sr ratio in vegetation can be altered by selective uptake or allocation within tissues, which leads to the regression line intercepting the x-axis somewhere other than the exact Ca/Sr value for wollastonite. The value provides an estimate required for calculating the DF. Error for each discrimination factor was calculated by adding and subtracting the standard error of the regression to or from the intercept and calculating the DF again (eq. 2). The difference of the two error DFs was divided by two to calculate the error for each DF calculation. Other statistical analysis was conducted using the PSAW ANOVA wizard after checking all valid assumptions for that test.

Discrimination Factor

$$1.\quad \text{(a) } DF = \frac{Ca/Sr_{proj}}{Ca/Sr_{uptake}} \qquad \text{(b) } DF_{woll} = \frac{Ca/Sr_{proj}}{Ca/Sr_{woll}} \qquad \text{(c) } DF_{stream} = \frac{Ca/Sr_{proj}}{Ca/Sr_{stream}}$$

Where $Ca/Sr_{proj} = \dfrac{\left(\dfrac{^{87}Sr}{^{86}Sr_{uptake}}\right) - y_i}{m}$, $DF = \dfrac{\dfrac{\left(\dfrac{^{87}Sr}{^{86}Sr_{uptake}}\right) - y_i}{m}}{Ca/Sr_{uptake}}$,

$Ca/Sr_{woll} = 2870$ and $Ca/Sr_{stream} = 3151$.

Error

$$1\sigma DF = \frac{\dfrac{\left(\dfrac{^{87}Sr}{^{86}Sr}\right) - (y_i \pm \sigma)}{m}}{Ca/Sr_{uptake}} \qquad 1\sigma DF = \frac{\dfrac{\dfrac{\left(\dfrac{^{87}Sr}{^{86}Sr}\right) - (y_i + \sigma)}{m}}{Ca/Sr_{uptake}} - \dfrac{\dfrac{\left(\dfrac{^{87}Sr}{^{86}Sr}\right) - (y_i - \sigma)}{m}}{Ca/Sr_{uptake}}}{2}$$

Results

Foliar Chemistry

Ash-Dasch et al. observed distinct changes in pre- and post-application values for foliar Ca/Sr and ^{87}Sr/^{86}Sr ratios. This chemical data is compiled in tables 3 and 4. With the addition of data from 2005-2008 no species has yet returned to Ca/Sr or ^{87}Sr/^{86}Sr values equal to those prior to application. However, many species display a 'rebound' effect as Ca/Sr and Sr87/87 begin to trend back to pre-application values. This 'peak' and 'rebound' effect is more strongly visible in Ca/Sr values than ^{87}Sr/^{86}Sr . Linear regression of ^{87}Sr/^{86}Sr ratios over time showed statistically significant trends for every species when data was aggregated from every elevation. Within a species, some individual elevation categories do not show significant trends (table 1). These were spruce 'old', sugar maple 'upper'; most likely due to small sample sizes, and wood fern 'high' due to a very strong peak and decrease seen in the data (figure 2). This confirms general downward trends in ^{87}Sr/^{86}Sr values over time. ANOVA was run on the data to confirm if elevation affects either Ca/Sr or ^{87}Sr/^{86}Sr for a species. There is an elevation effect, but it is stronger for Ca/Sr than ^{87}Sr/^{86}Sr . Every species but spruce showed significance for Ca/Sr trends, but only wood fern showed a significant trend for ^{87}Sr/^{86}Sr.

Table 1
ANOVA of 87Sr/86Sr and Ca/Sr
Test significance of elevational differences

	p-value		
	87/86	Ca/Sr	n
Beech	0.646	0.019	40
Fir	0.153	0.225	20
Spruce	0.068	0.197	25
Sugar Maple	0.419	0.28	34
Viburnum	0.448	0.003	39
Wood Fern	0.019	>.0001	39

Table 2

Linear Regressions of $^{87}Sr/^{86}Sr$ and time 1999-2008

Species		r2	p-value	n
Beech	All	0.668	<.001	40
	Low	0.754	0.001	10
	Mid	0.713	<.001	15
	Upper	0.76	0.001	10
	High	0.95	0.031	5
Spruce	All	0.516	<.001	25
	New	0.576	<.001	15
	Old	0.398	0.129	7
Sugar Maple	All	0.717	<.001	34
	Low	0.868	<.001	15
	Mid	0.896	<.001	11
	Upper	0.49	0.507	3
	High	0.364	0.281	5
Fir	All	0.725	<.001	20
	New	0.734	<.001	10
	Old	0.736	0.014	7
Viburnum	All	0.512	<.001	39
	Low	0.723	0.002	10
	Mid	0.651	0.009	9
	Upper	0.584	0.01	10
	High	0.595	0.009	9
Wood Fern	All	0.236	0.002	40
	Low	0.541	0.015	10
	Mid	0.574	0.011	9
	Upper	0.564	0.012	10
	High	0.118	0.331	9

Rebound of Ca/Sr and $^{87}Sr/^{86}Sr$ after application

All species, with the exception of spruce, experienced a peak in the Ca/Sr ratio a few years after application. As figure 1 shows, for wood fern (all elevation data), the lowest $^{87}Sr/^{86}Sr$ and highest Ca/Sr values occurred in 2003 and 2004. Data for years after that only confirmed the general trend already apparent in the data towards the chemical values from the wollastonite. The range in peak Ca/Sr value is substantial, going from viburnum at 1101, to a maximum value of 4033 in Spruce. Peaks in Ca/Sr were generally observed at higher elevations. The lowest Ca/Sr ratios were all observed pre-application in 1999 and mostly at low elevations. Again spruce is an exception to this trend. The Ca/Sr ratio for spruce appears to jump up and down quite dramatically (up to 200%) in the nine years sampled. The highest Ca/Sr value was measured prior to application. Ca/Sr values trend lower over time, but still contain a lot of variability.

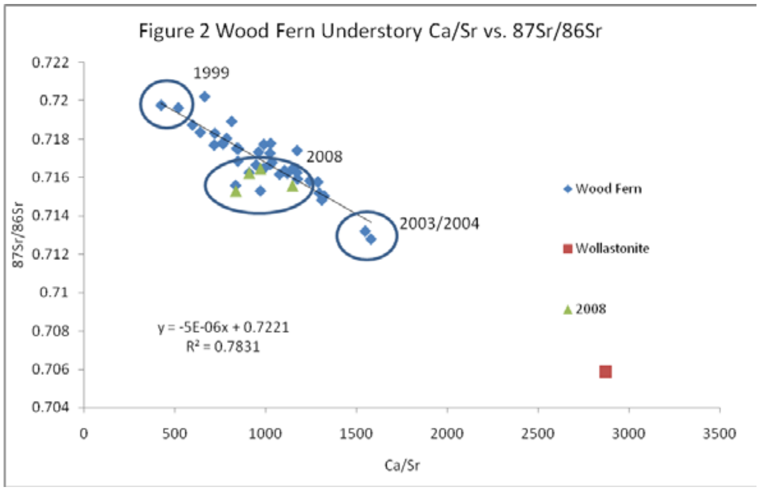
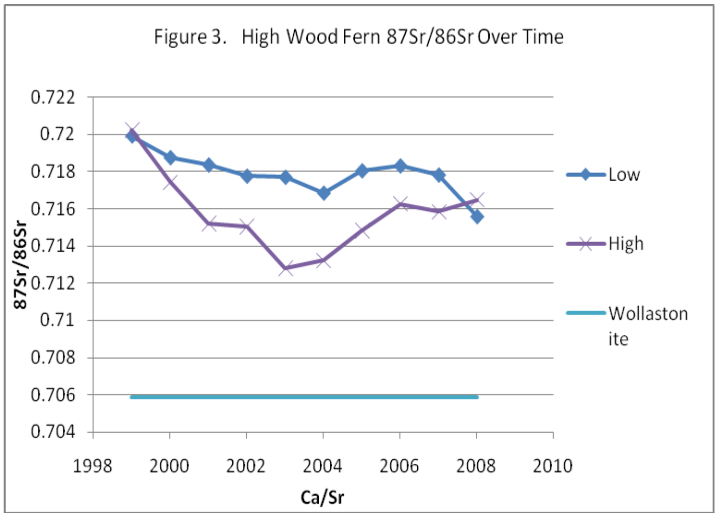


Figure 2 Wood Fern Understory Ca/Sr vs. 87Sr/86Sr

Fir, another conifer, in the Pinaceae family, shows similar but less extreme variability in Ca/Sr ratio, but does have an overall upward trend with time, like other species measured, where spruce shows a general downward trend in Ca/Sr values. The two understory species



measured, viburnum and wood fern experienced the peak earlier, 2005 and 2003 respectively. This may be due to the fact that these two species are less complex, especially the fern, than the trees. Beech and yellow birch, two hardwood trees, experienced peaks in Ca/Sr ratio six years after application. The magnitude of the peak were also similar, making it seem likely that the two species handle calcium and strontium similarly to each other, although the total number of data points and the consistency of the data varies between the two species. Sugar maple behaves quite differently, not much variation is seen in yearly Ca/Sr values at low and mid elevations which both peak in 2003. High and upper zones show different, less consistent trends, but limited or inconsistent data for these ranges makes interpretation difficult. Those that have peaked already appear to be returning to pre-application Ca/Sr values. Fir may peak in the next few years and then begin to return as well, but this is not visible in the our range of data. Spruce may reach a low in Ca/Sr and bounce back, but general variability in the observed values makes this prediction less likely.

Table 3. Ca/Sr Maximums and Minimums 1999-2008

	Maximum	Year	Elevation	Minimum	Year	Elevation
Beech	1735	2006	Upper	748	1999	Low
Fir	3459	2007	New	1570	2002	New
Spruce	4033	1999	New	1462	2008	New
Sugar Maple	1213	2003	Upper	432	1999	Mid
Viburnum	1101	2005	High	394	1999	Low
Wood Fern	1580	2003	High	423	1999	Low
Yellow Birch	1250	2006	High	602	1999	Low

^{87}Sr/^{86}Sr measured values tend to shift towards the ratio of the applied wollastonite (.70588) with time, with slight 'rebound' appearing in the most recent data, depending on the species (see table 3). ^{87}Sr/^{86}Sr values did not show the initial variation related to elevation that Ca/Sr ratios did. The range of values between elevations was also much smaller than the range observed in Ca/Sr ratios. Beech, fir, viburnum, and wood fern experienced minimum ^{87}Sr/^{86}Sr ratios in the same year and mostly the same elevation as the peak in Ca/Sr. Sugar maple dosen't have a complete data set for the entire time for upper and high elevations, so estimates of peak wollastonite absorption are flawed for this species. Limited data also hinders estimates for yellow birch. The high variability seen in every measurement for spruce makes seeing or assessing any trend difficult or impossible.

Table 4. $^{87}Sr/^{86}Sr$ Maximums and Minimums 1999-2008

	Minimum	Year	Elevation	Maximum	Year	Elevation
Beech	0.7175	2006	High	0.7207	2000	Upper
Fir	0.7155	2007	New	0.7200	2001	New
Spruce	0.7158	2006	New	.7210	2001	Old
Sugar Maple	0.7155	2008	Mid	.7219	2000	Upper
Viburnum	0.7155	2005	High	.7210	2000	Mid
Wood Fern	0.7128	2003	High	0.7202	1999	High
Yellow Birch	0.7186	2000	Low	.7201	1999	Mid

Regressions

Regressions of Ca/Sr vs 87Sr/86Sr for every species and elevation class or needle class are shown in figures 4 and 5. Some regressions were more powerful than others, and some elevation classes are different enough within a species that combining them into an “all” category is not effective or statistically powerful enough to use for discrimination factor calculations. The results for all classes are reported anyway.

Discrimination Factors

Discrimination factors were calculated separately for overstory and understory data by species further sub divided elevation. The resulting data is compiled in Table 5 for the understory, and table 6 for the overstory. With the data added since 2004, regressions for every species were significant and grouping together all beech elevation zones to achieve a significant regression—as Ash-Dasch et al. did—was unnecessary.

Changes in DF since 2004

For understory data, error decreased with the addition of data taken since 2004. However there is a broad range of observed changes to DF between the two data sets (figure 6 4). For beech “All” DF_{woll} went down 30%, where wood fern “All” only changed by 2%. Data for understory sugar maple at the upper elevations only consisted of measurements taken from 1999-2001, so no change in DF is observable. The small amount of change in DF_{woll} for wood fern reinforces any conclusions made about the species behavior with regard to calcium made by Ash-Dasch et al. (2006).

Figure 4 Ca/Sr vs 87Sr/86Sr regressions for understory data at each elevation or needle class.

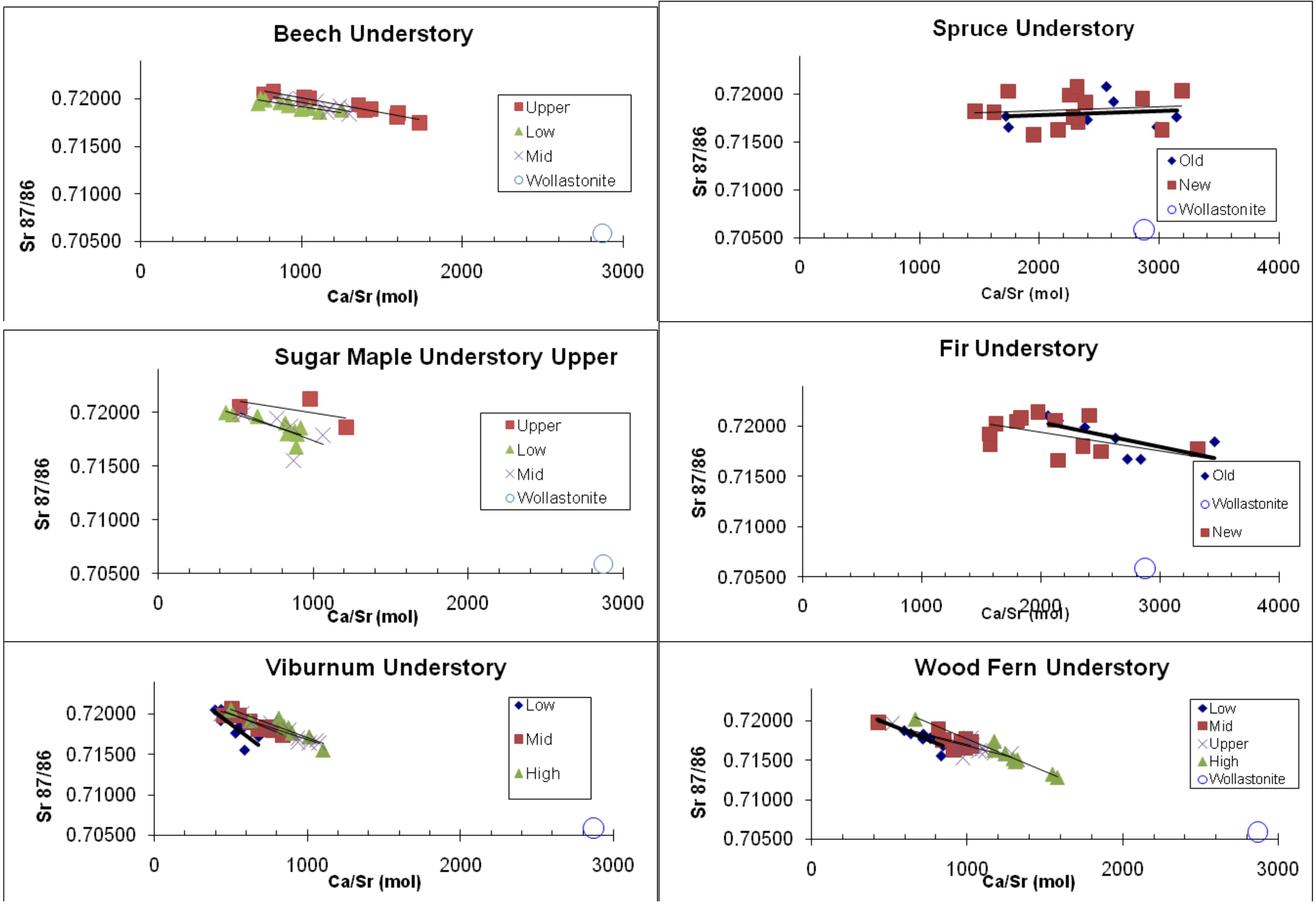


Figure 5. Ca/Sr vs 87Sr/86Sr regressions for overstory data at each elevation or needle class.

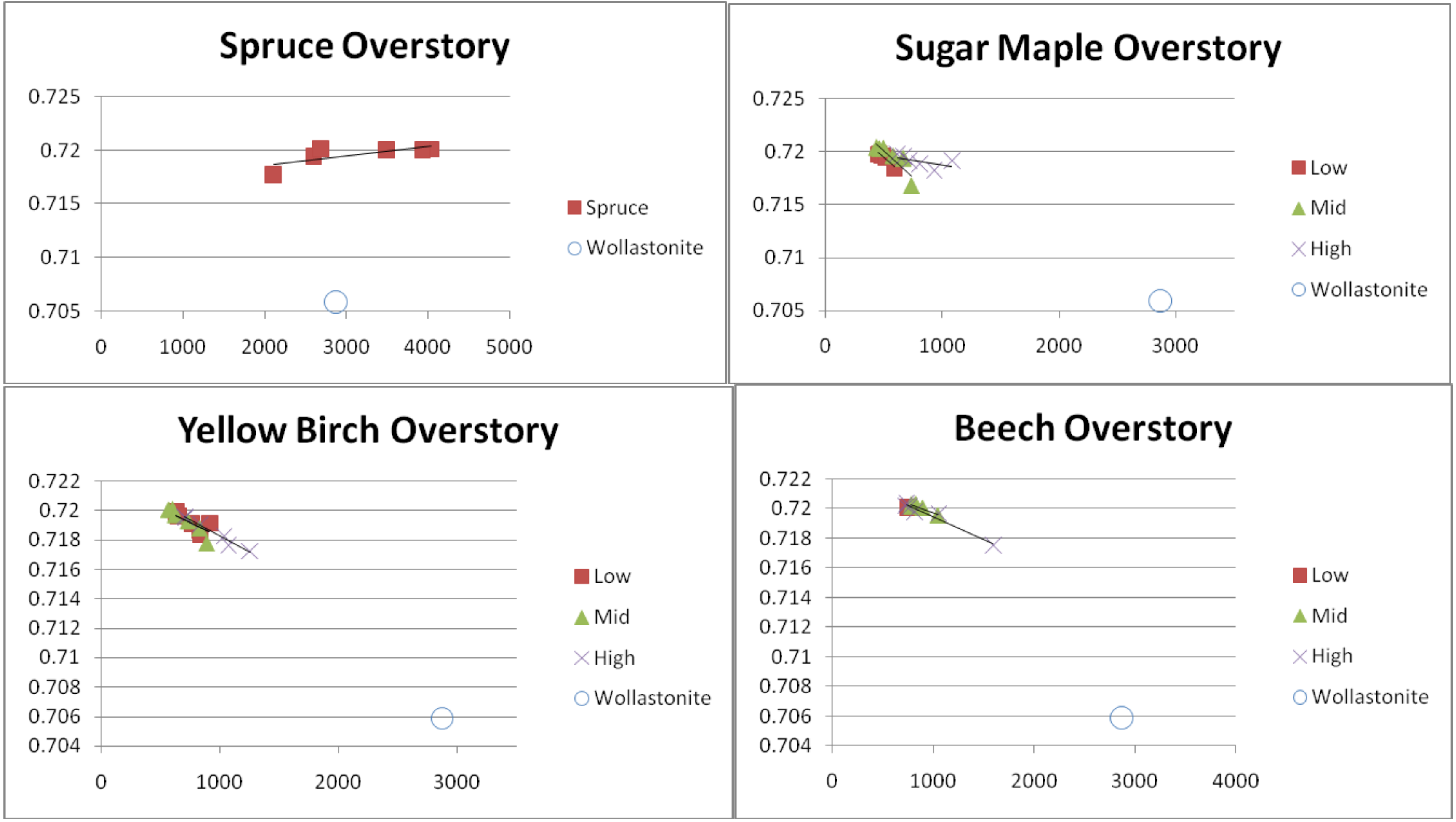
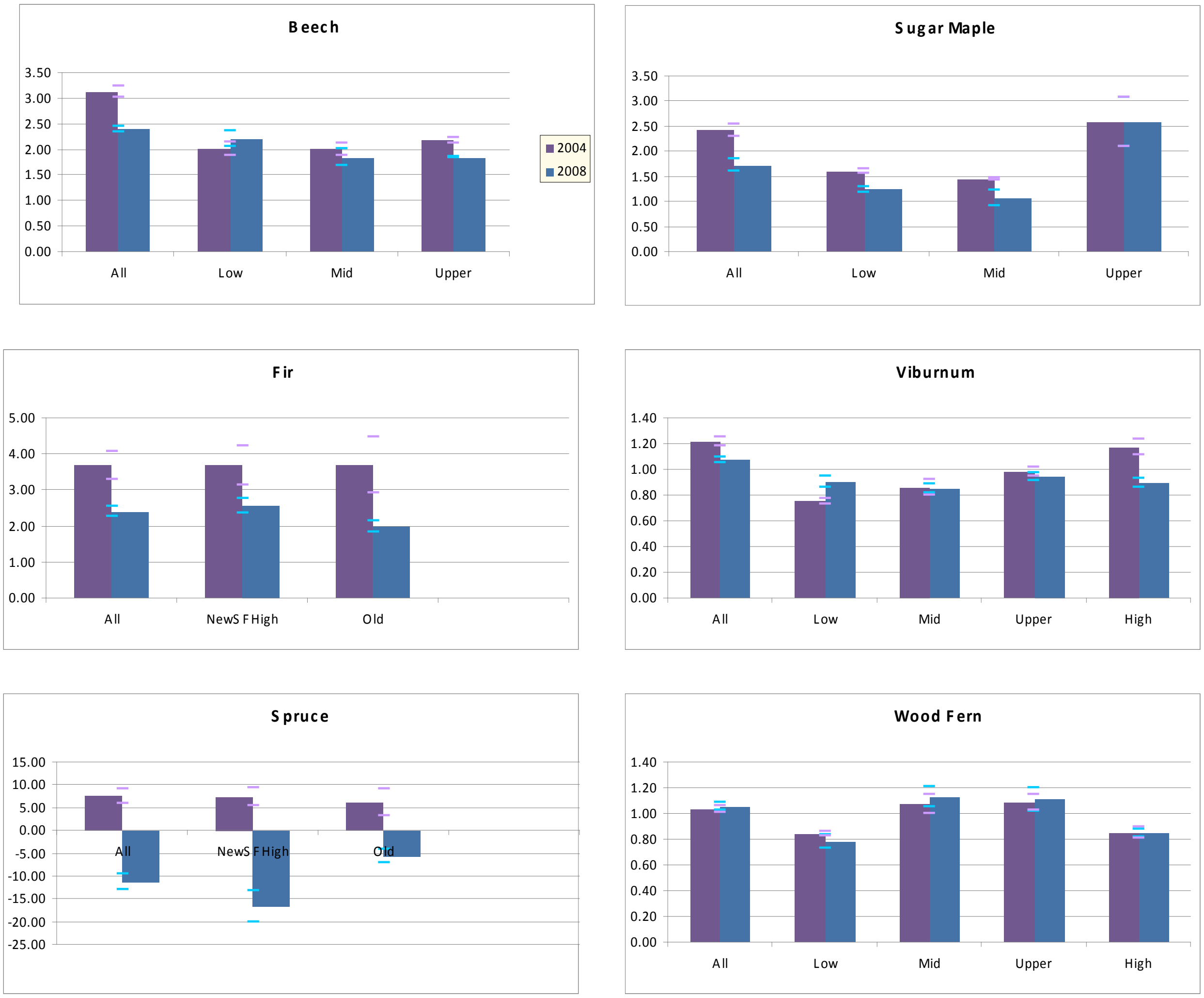


Figure 6. Understory DFwoll calculated from data up to 2004 and 2008 with error bars.



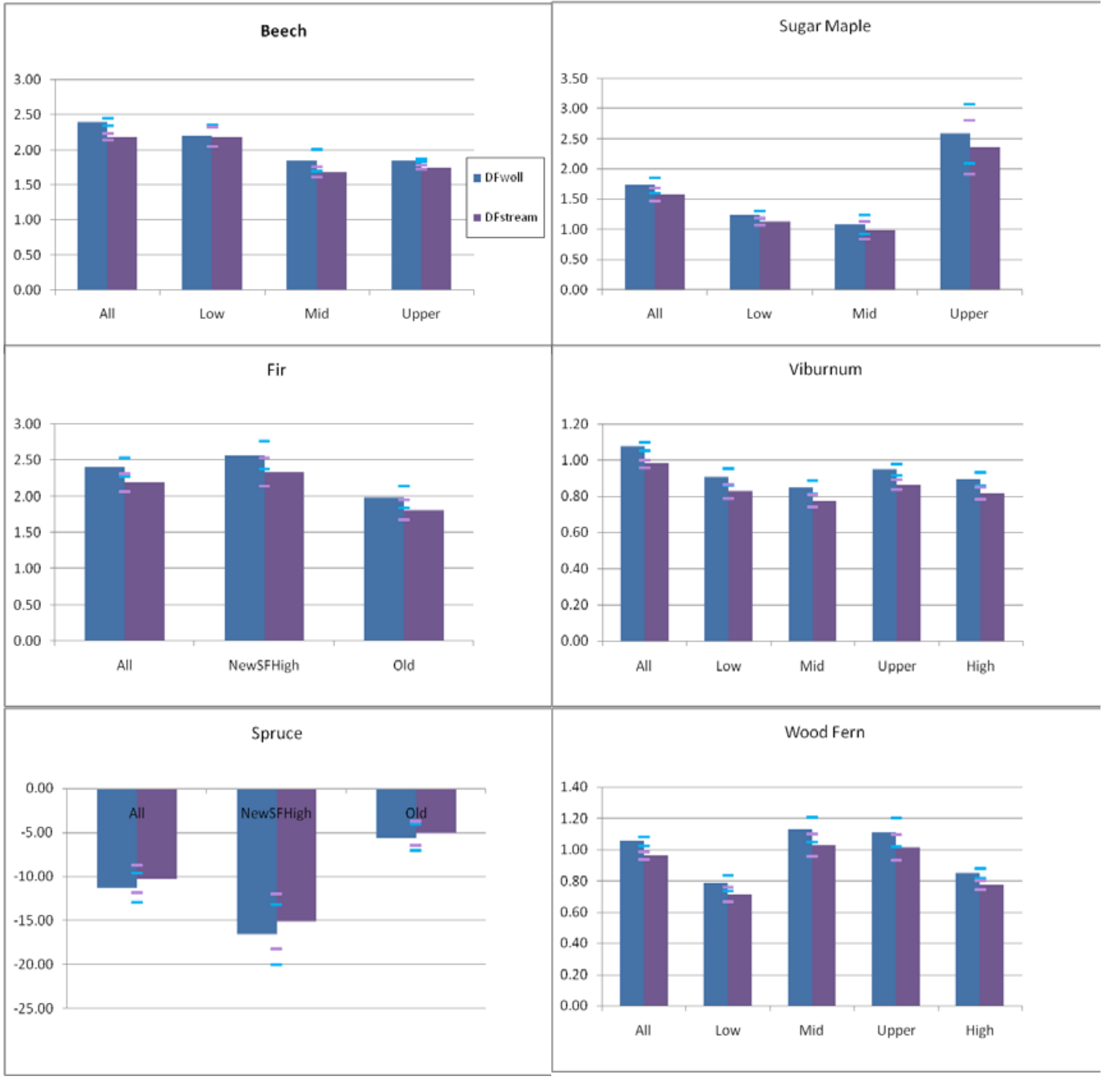
DF$_{woll}$ and DF$_{stream}$

The calculation for DF$_{stream}$ (the discrimination factor based on the Ca/Sr value of stream water see eq. 1) necessarily returns a DF lower than DF$_{woll}$. Calculated error for DF$_{stream}$ is generally smaller by about 0.01 (See error eq.2). For most species DF$_{woll}$ and DF$_{stream}$ were distinct, and error bars for either calculation did not overlap (figure 6 5). For high and new fir, spruce at every elevation, upper wood fern, and low yellow birch, the errors for DF$_{woll}$ and DF$_{stream}$ did intersect, but these categories have wider error envelopes, indicating less certainty about the calculation overall.

Overstory vs. Understory

Inconsistencies in data between overstory and understory make it difficult to compare the two categories in terms of chemistry and in DF. Between the two data sets, sample sizes are not the same, data is missing for the years between 2004 and 2005 for overstory, and some species were not sampled in the overstory and understory for every elevation class. Beech was one of the few species with fairly consistent overstory and understory data. Figure 7 below shows regressions of overstory and understory Ca/Sr vs $^{87}Sr/^{86}Sr$ data. Only overstory samples were taken for yellow birch, so no understory/overstory comparison can be made. As was seen in the understory, updated DF calculations are generally distinct within error from DF's calculated with data from between 1999-2003 and error envelopes for 2006 DF's are generally narrower than their 2003 counter parts. Because this data set only included one

Figure 7. DF$_{woll}$ and DF$_{stream}$ calculated from understory data 1999-2008.



new point since 2003, we may be introducing more error into the calculations. Our sporadic data may be missing the peaks in Ca/Sr and $^{87}Sr/^{86}Sr$ values that defined the overall trends towards wollastonite observed for species with more complete data.

Figure 8.Overstory DF_{woll} calculated from data up to 2003 and 2006 with error bars.

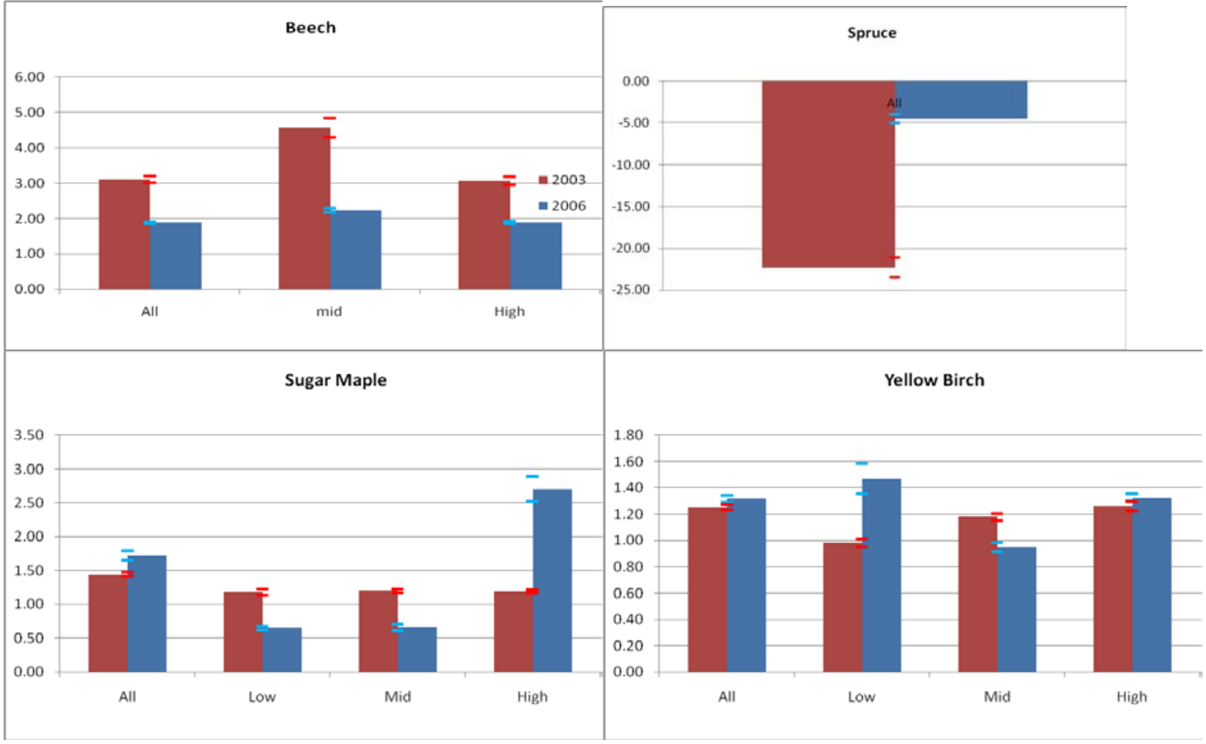
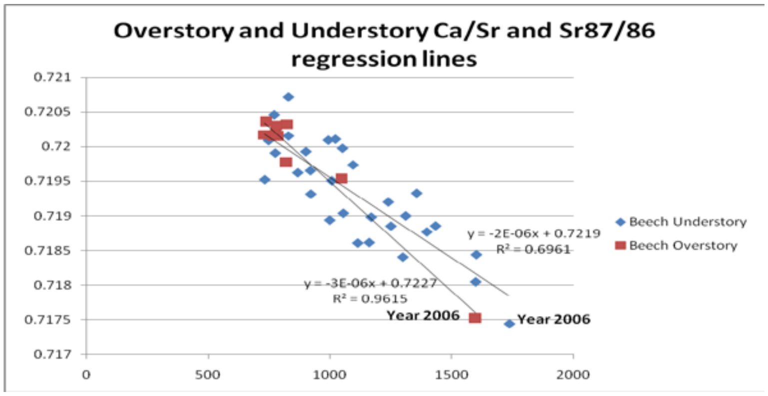


Figure 9

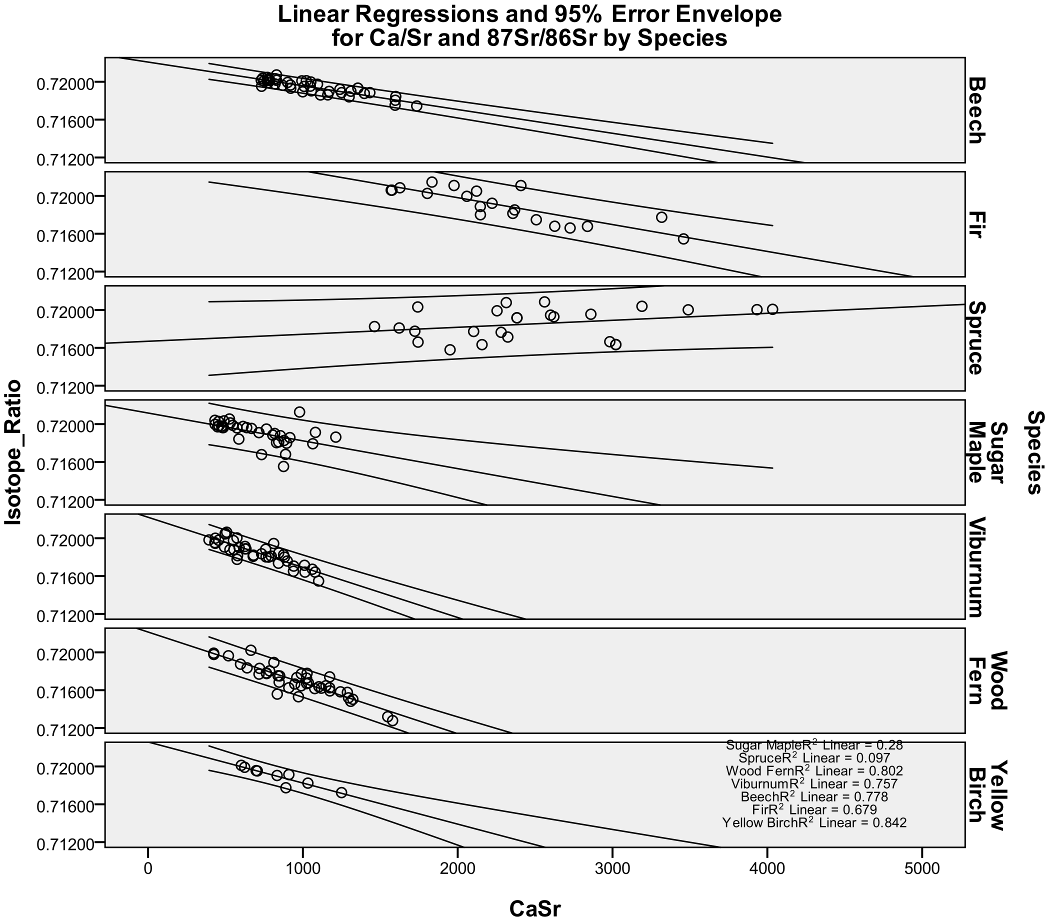


Discussion

Discrimination Factors

The DF calculation depends on extrapolating a regression line beyond the range of the data. Statistically this is not the most powerful projection, but is useful here to relate the trend seen in the data to the Ca/Sr and ^{87}Sr/^{86}Sr values in the source wollastonite. We attempted to be aware of the error we were incorporating into our calculations by calculating error for discrimination factors with the error of the slope included. The total error of the regression can be seen in these 95% confidence interval error envelope graphs (figure 10). The discrimination factor calculated with a Ca/Sr source value from stream water was conducted to independently check DF$_{woll}$ calculation. By virtue of their calculations, DF$_{woll}$ and DS$_{stream}$ were different, with small differences in error depending on the species and elevation measured. In many cases the two values were within error of each other.

Figure 10.



Changes in DF since 2004

Small variations between my DFs recalculated for 1999-2004 and those published by Ash-Dasch et al. are likely due to differences in grouping data by elevation or by differences in rounding raw numbers done by the regression program within MS Excel or PSAW (formerly SPSS). Ash-Dasch et al. do not specify if the DF's published are for understory or overstory samples. Ash-Dasch et al. also did not publish data or DF's for beech trees at low elevations—however, we now report these data. The authors did not calculate DF's for fir or spruce, most likely because the data didn't show clear trends related to the addition of wollastonite.

With the addition of data from 2004-2008 DF's for most species changed considerably. Where comparisons can be made to figures published in Ash-Dasch et al. 2006 only two of the initial DFs fall within the error envelope calculated for DFs since 2004. These were DF_{woll} for viburnum upper, and DS_{stream} for viburnum low, indicating that either the wollastonite's influence on the chemistry of these plants at these elevations had already peaked and the general trend in the data had been established, or that new data only confirmed the pattern already observed.

The difficulty in comparing somewhat mismatched data led us to re-calculate DFs after removing data for 2005-2008 (for understory) and 2006 (for overstory) to better assess changes in calculated DF (figure 3). Because of the similarity between DF_{woll} and DS_{stream}, these comparisons were made only using DF_{woll} from 1999-2004 and 1999-2008, or 2006 for overstory, presuming that any changes between the time period for DF_{woll} would only be repeated by DS_{stream}.

DF_{woll} for wood fern "All" was calculated at $1.06 \pm 0.03\ \sigma 1$. Wood fern appears to be a model species" and possibly a good indicator of short term changes in calcium across an ecosystem. The large amount of data and its consistency means that the regression was very powerful (r2=0.80 for "All"). Very little change was seen in DF values between 2004 and 2008. This is likely due to the fact that this species was already returning to pre-application Ca/Sr levels by 2004 and the pattern represented by the linear regression was already 'set' in the data. Some altitudinal variation in DF is evident. Low altitude samples indicate a lower DF than higher altitudes. Wood fern's sensitivity to calcium addition may be due to the fact that it is neither an angiosperm nor a woody plant. It may simply access calcium and strontium from soil and water into its tissues more efficently than more complex plants.

Beech has the most thorough and consistent data set for any tree sampled. Data was available for both overstory and understory samples. Beech is in the same taxonomical family as oaks and chestnuts. Compared to DF's from 2004, there was a general decrease in DF value with the added data. However, the error envelopes for low and mid elevations overlap for 2004 and 2008 indicating there was not much difference between the two values. DF_{woll} for "All" was $2.40 \pm 0.05\ \sigma 1$, and it hovered around 2 for the other elevations measured. Because a discrimination factor greater than one indicates preferential uptake of Ca over Sr, these values indicate a strong preference for calcium in the foliage of beech trees. Beech DFs could be used to estimate the preference of other hardwood trees, but comparisons should be made to other members of the fagaceae family before using the beech DF for other species.

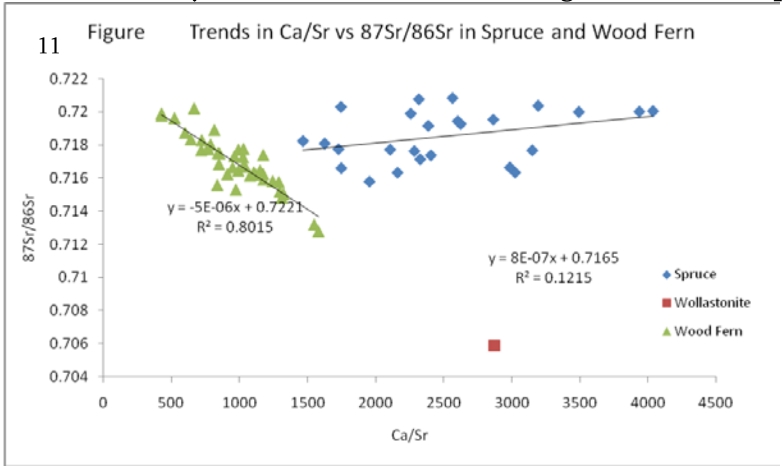
The sugar maple (sapindaceae family) dataset is not as robust as the other hardwood species sampled. Samples were not taken for every elevation zone each year, making conclusions about its behavior between elevation zones difficult or impossible. In some cases this is likely due to the scarcity of certain species at high elevations that are common at lower elevations. Researchers were perhaps able to locate a single sugar maple above 670m in some years, and failed to do so in other years. However there are still apparent trends and inferences that can be made. Low and mid elevations showed a decrease in DF since 2004 data was analyzed. Both elevation classes have DF's near 1.24 with error. Because of the small number of data points available (n=3) for upper elevation sugar maple, these two, low and mid elevations, may be representative of the tree's behavior with respect to Ca and Sr. Data for upper elevation samples was only taken from 1999-2001, before other trees began to be influenced much by the wollastonite addition. Because of this the DF calculated from this 'upper' data may be entirely inaccurate or give an overestimate of DF.

Viburnum is a woody shrub common to most northern forests. It is in the Adoxaceae family with elders and honeysuckles. This species also has consistent and complete data for the entire nine year span of measurements. Because both viburnum and wood fern are understory plants without the complex structure of trees, the two species may behave similarly with respect to calcium absorption. Viburnum shows a similar increasing trend in DF with elevation to wood fern. Viburnum and wood fern also experienced the earliest peaks in Ca/Sr ratio; in 2003 and 2005 respectively.

Yellow birch (betulaceae family) was only sampled in the overstory. No data was available between 2003 and 2006. New DF values were calculated including the new value for 2006 for each elevation zone but the regression for this particular species is not as strong as it could be considering the missing data. DF's for yellow birch were between 1.1 and 1.3 for every elevation category. These values are closer to wood fern and viburnum DF's and may indicate that yellow birch behaves more like the understory species with respect to calcium than like the other two hardwoods (beech and maple) which were analyzed.

The two conifer species sampled were fir (genus Abies) and spruce (genus Picea) present only in the highest reaches of the experimental watershed. DF_{woll} for new fir needles was 2.31 ± 0.18 $\sigma 1$, and 1.99 ± 0.15 $\sigma 1$ for old needles. These two values overlap within error, but there may be differential incorporation of Ca and Sr based on the age of needles. If this is the case it would be useful to know whether there is a biological mechanism excluding Sr from incorporation into new needles that is less effective in older needles. Overall DF's for fir were higher than many other species. This indicates again a preference for calcium over strontium. DF's calculated with data from 1999-2004 resulted in the highest DF's found around 3.5 for each species. DF's from 1999-2008 data were lower, but still greater than every other species with the exception of spruce which is generally anomalous.

Despite fairly robust sample sizes, the spruce data produced no regressions with r2 values above 0.31. The data had slightly positive slopes where every other species displayed much more strongly negative slopes trending towards the wollastonite value. Using the equations above with this slope yielded large and negative DFs. DFwoll for spruce "All" was 4.61 ± 1.11 $\sigma 1$. No other species had errors larger than 1. Qualitatively the data show very little variation in $^{87}Sr/^{86}Sr$ ratios, and a wide but apparently trendless variation in Ca/Sr ratios (figure 11). Because spruce is very sensitive to calcium depletion (DeHayes 1999) being able to trace calcium sources and monitor depletion effects would be very usefull to the preservation of spruce in Northeastern forests. However, the data suggest that spruce may not take up strontium in a way that is characteristic enough to trace isotopic ratios.


11 Figure Trends in Ca/Sr vs 87Sr/86Sr in Spruce and Wood Fern

The taxonomic variety of species sampled gets a good picture of how calcium travels through this specific ecosystem, but makes making species level generalizations about calcium and strontium uptake difficult. Huge variation was observed between the seven species sampled in DF, Ca/Sr and ^{87}Sr/^{86}Sr behavior. Some of this variation can be explained at the species level. Wood fern showed the earliest peak in Ca/Sr and the largest difference in values between pre- and immediately post-application possibly because it is a relatively simple plant without the cellular complexity of the hardwoods sampled. This might also explain why it is generally “better” behaved than other more complex species.

This experiment is ongoing and new samples are collected every year. Overstory data for 2007-2009 was received earlier this year but was not analyzed in time to be included in this paper. If the ‘peak and rebound’ effect observed for Ca/Sr values in many species is valid, newer data will confirm that foliar chemistry is returning to pre-application values. In the next five or ten years all species should have returned to pre-application or close to pre-application chemistry, which will indicate the end of the added wollastonite’s effect on the large plants in the ecosystem. After this point, further measurement of foliar chemistry of trees may not be useful to further monitoring the effects of calcium addition and it may be more interesting to focus on any propagation of the source wollastonite into other plants or nutrient pools within the ecosystem.

Table 3

Understory Chemistry

Species	Date	Ca mg/g	Sr mg/g	Ca/Sr mol	87Sr/86Sr
Beech	1999	4.80	0.01403	748	0.720092
Low	2000	6.10	0.01818	733	0.719526
	2001	5.28	0.01489	776	0.719911
	2002	7.22	0.01820	868	0.719626
	2003	6.06	0.01440	920	0.719656
	2004	7.47	0.01634	999	0.718941
	2005	5.98	0.01240	1055	0.719040
	2006	7.17	0.01702	921	0.719318
	2007	5.87	0.01152	1114	0.718608
	2008	3.26	0.00571	1250	0.718854
Beech	1999	4.71	0.01142	901	0.719932
Mid	2000	5.84	0.01538	830	0.720159
	2001	5.19	0.01142	993	0.720098
	2002	6.43	0.01284	1095	0.719739
	2003	6.05	0.01314	1008	0.719510
	2004	7.30	0.01365	1169	0.718984
	2005	6.16	0.01029	1310	0.719002
	2006	7.23	0.01217	1299	0.718407
	2007	6.51	0.01227	1161	0.718616
	2008	2.98	0.00525	1239	0.719202
Beech	1999	4.37	0.01239	772	0.720464
High	2000	4.83	0.01272	830	0.720725
	2001	5.41	0.01158	1022	0.720112
	2002	6.68	0.01390	1051	0.719980
	2003	6.18	0.00997	1355	0.719331
	2004	6.60	0.01033	1397	0.718770
	2005	6.21	0.00848	1600	0.718442
	2006	7.83	0.00986	1735	0.717441
	2007	6.42	0.00878	1597	0.718048
	2008	3.59	0.00547	1433	0.718857

Species	Date	Ca mg/g	Sr mg/g	Ca/Sr mol	^{87}Sr/^{86}Sr
Fir	1999	4.95	0.00600	1804	0.720262
New	2000	5.16	0.00694	1627	0.720857
	2001	4.60	0.00548	1834	0.721460
	2001	3.53	0.00321	2408	0.721095
	2002	7.67	0.01068	1570	0.720604
	2002	6.47	0.00666	2122	0.720502
	2003	5.00	0.00464	2356	0.718152
	2004	4.02	0.00395	2222	0.719220
	2005	8.74	0.00890	2147	0.718006
	2006	5.28	0.00424	2726	0.716607
	2007	4.75	0.00300	3459	0.715453
	2008	4.73	0.00412	2508	0.717479
	2008	2.30	0.00151	3318	0.717742
Fir	2001	6.78	0.00750	1976	0.721098
Old	2002	6.81	0.00945	1575	0.720603
	2003	8.55	0.00871	2146	0.718880
	2004	9.51	0.01009	2059	0.719956
	2005	4.26	0.00355	2627	0.716793
	2006	10.08	0.00931	2368	0.718518
	2007	10.80	0.00832	2838	0.716777
Spruce	1999	4.88	0.00613	1742	0.720319
New	2000	2.92	0.00284	2254	0.719917
	2001	2.43	0.00229	2314	0.720782
	2001	1.42	0.00098	3190	0.720391
	2002	4.33	0.00397	2383	0.719173
	2003	1.09	0.00079	3021	0.716345
	2004	1.58	0.00160	2157	0.716339
	2005	3.71	0.00349	2324	0.717143
	2006	1.81	0.00203	1951	0.715794
	2007	1.83	0.00175	2281	0.717642
	2008	2.84	0.00383	1622	0.718100
	2008	0.74	0.00111	1463	0.718251
	2002	3.46	0.00265	2860	0.719552

Table 3
Understory Chemistry continued

Species	Date	Ca mg/g	Sr mg/g	Ca/Sr mol	87Sr/86Sr
Spruce	2001	3.93	0.00336	2561	0.720856
Old	2002	1.66	0.00139	2621	0.719283
	2003	3.71	0.00258	3147	0.717684
	2004	3.68	0.00335	2402	0.717387
	2005	1.21	0.00089	2982	0.716648
	2006	4.34	0.00544	1744	0.716607
	2007	3.90	0.00495	1723	0.717757
Sugar Maple	1999	6.56	0.03316	433	0.719977
Low	2000	8.56	0.03936	476	0.719788
	2001	8.86	0.03041	637	0.719628
	2002	10.92	0.02920	818	0.719010
	2003	9.73	0.02321	916	0.718572
	2004	10.80	0.02638	895	0.717987
	2005	8.26	0.02057	878	0.718270
	2006	10.71	0.02775	844	0.718117
	2007	10.32	0.02720	830	0.718036
	2008	6.30	0.01551	888	0.716801
Sugar Maple	1999	5.99	0.02392	547	0.719870
Mid	2000	9.46	0.03893	531	0.720129
	2001	8.82	0.02257	855	0.718775
	2002	11.75	0.03359	765	0.719477
	2003	9.28	0.01907	1064	0.717925
	2008	6.25	0.01561	875	0.715523
Sugar Maple	1999	5.59	0.02326	526	0.720540
Upper	2000	4.92	0.01099	979	0.721276
	2001	7.87	0.01420	1213	0.718625

Species	Date	Ca mg/g	Sr mg/g	Ca/Sr mol	^{87}Sr/^{86}Sr
Viburnum	1999	8.97	0.04985	394	0.719818
Low	2000	10.59	0.05339	434	0.719459
	2001	10.01	0.05075	431	0.719535
	2002	13.15	0.05823	494	0.719068
	2003	11.28	0.04451	554	0.718812
	2004	11.64	0.04404	578	0.718156
	2005	10.61	0.03940	588	0.719010
	2006	12.92	0.04149	681	0.718208
	2007	9.51	0.03931	529	0.718793
	2008	5.16	0.01962	575	0.717766
Viburnum	1999	9.00	0.04319	456	0.719783
Mid	2000	11.56	0.04976	508	0.720634
	2001	12.12	0.04807	551	0.719756
	2002	14.71	0.05133	626	0.718871
	2003	12.33	0.04329	623	no signal
	2004	12.76	0.03584	778	0.718003
	2005	12.70	0.03298	842	0.717359
	2006	12.98	0.03567	796	0.718102
	2007	12.51	0.04030	679	0.718087
	2008	5.65	0.01686	733	0.718354
Viburnum	1999	7.74	0.03898	434	0.720001
High	2000	11.70	0.04455	574	0.720033
	2001	11.93	0.04124	632	0.718877
	2002	15.38	0.04427	759	0.718832
	2003	11.98	0.02783	941	0.717055
	2004	7.01	0.01420	1079	0.716420
	2005	11.53	0.02685	939	0.716509
	2006	16.94	0.03654	1013	0.716418
	2007	13.88	0.02859	1061	0.716722
	2008	5.56	0.01596	762	0.718024

Table 3
Understory Chemistry continued

Species	Date	Ca mg/g	Sr mg/g	Ca/Sr mol	87Sr/86Sr
Viburnum	1999	16.56	0.07272	498	0.720455
High	2000	11.35	0.04977	498	0.720512
	2001	13.54	0.04727	626	0.719132
	2002	15.08	0.04064	811	0.719441
	2003	12.48	0.03232	844	0.718439
	2004	12.35	0.03086	875	0.718244
	2005	11.99	0.02380	1102	0.715476
	2006	15.77	0.03412	1010	0.717138
	2007	11.73	0.02853	899	0.717588
	2008	6.54	0.01623	881	0.718000
Wood Fern	1999	3.95	0.02036	424	0.719911
Low	2000	5.18	0.01897	597	0.718747
	2001	4.44	0.01518	640	0.718356
	2002	4.52	0.01291	766	0.717756
	2003	4.44	0.01353	717	0.717697
	2004	4.09	0.01056	847	0.716847
	2005	3.51	0.00977	785	0.718041
	2006	4.11	0.01248	720	0.718304
	2007	3.78	0.01080	764	0.717817
	2008	1.80	0.00471	834	0.715585
Wood Fern	1999	3.25	0.01672	425	0.719774
Mid	2000	5.31	0.01428	813	0.718925
	2001	5.34	0.01216	961	0.717331
	2002	4.61	0.01185	850	0.717507
	2003	4.37	0.01139	840	0.717522
	2004	4.28	0.00947	989	0.716463
	2005	3.62	0.00772	1025	0.716693
	2006	4.46	0.00985	990	0.717726
	2007	3.78	0.00807	1024	0.717274
	2008	1.62	0.00390	909	0.716249
Wood Fern	1999	3.10	0.01305	519	0.719634
Upper	2000	6.61	0.01407	1027	0.717778
	2001	5.82	0.01182	1076	0.716168
	2002	6.11	0.01194	1119	0.716212
	2003	4.45	0.00940	1035	0.716774
	2004	4.88	0.00828	1287	0.715769
	2005	4.08	0.00942	947	0.716651
	2006	5.14	0.01019	1104	0.716360
	2007	3.92	0.00729	1175	0.715922
	2008	1.94	0.00437	971	0.715304
Wood Fern	1999	2.64	0.00871	664	0.720218
High	2000	5.16	0.00962	1173	0.717407
	2001	5.32	0.00898	1295	0.715189
	2002	4.73	0.00781	1323	0.715036
	2003	3.72	0.00515	1580	0.712784
	2004	4.27	0.00603	1548	0.713198
	2005	3.96	0.00662	1309	0.714822
	2006	4.97	0.00926	1173	0.716254
	2007	3.96	0.00697	1242	0.715825
	2008	1.51	0.00288	1149	0.716472

Table 4
Overstory Chemistry
1999-2006

Species	Date	Ca mg/g	Sr mg/g	Ca/Sr mol	87/86
Beech	1999	8.63	0.02291	823	0.720320
Mid	2000	6.41	0.01795	781	0.720305
	2001	5.54	0.01540	786	0.720153
	2002	6.66	0.01874	777	0.720174
	2003	6.96	0.01700	893	0.720060
	2006	7.28	0.01519	1048	0.719536
Beech	1999	4.91	0.01468	731	0.720169
High	2000	5.16	0.01526	739	0.720364
	2001	5.35	0.01520	770	0.720151
	2002	7.16	0.01909	820	0.719771
	2003	6.18	0.01280	1056	0.719676
	2006	9.12	0.01249	1596	0.717525
Spruce	1999	1.57	0.00085	4034	0.720071
High	2000	4.49	0.00377	2600	0.719457
	2001	1.58	0.00088	3933	0.720037
	2002	2.55	0.00160	3489	0.720019
	2003	2.78	0.23000	2683	0.720145
	2006	1.92	0.00200	2103	0.717235
Sugar Maple	1999	5.72	0.02782	449	0.719712
Low	2000	6.13	0.02799	479	0.719638
	2001	7.93	0.03787	458	0.719820
	2002	9.35	0.04204	486	0.719629
	2003	8.22	0.03470	517	0.719445
	2006	6.30	0.02348	586	0.719126
Sugar Maple	1999	5.66	0.02865	432	0.720400
Mid	2000	5.37	0.02395	490	0.720341
	2001	7.39	0.03547	455	0.720282
	2002	14.03	0.05337	575	0.719600
	2003	10.21	0.03380	662	0.719377
	2006	9.65	0.02876	733	0.719137
Sugar Maple	1999	5.41	0.01929	613	0.719754
High	2000	4.62	0.01511	668	0.719544
	2001	6.41	0.01957	716	0.719101
	2002	11.47	0.03112	805	0.718842
	2003	10.03	0.02360	930	0.718180
	2006	9.88	0.01998	1081	0.717748
Yellow Birch	1999	9.14	0.03204	624	0.719909
Low	2000	7.95	0.02700	876	0.719593
	2001	8.21	0.02370	758	0.719111
	2002	10.80	0.02860	827	0.718638
	2003	13.54	0.03320	892	0.718377
	2006	13.54	0.03256	909	0.717730
Yellow Birch	1999	8.33	0.03024	602	0.72011
Mid	2000	7.74	0.02980	568	0.72009
	2001	7.58	0.02660	624	0.71972
	2002	9.93	0.02960	733	0.71929
	2003	11.43	0.03010	831	0.71881
	2006	15.56	0.03822	890	0.71842
Yellow Birch	1999	6.45	0.02019	698	0.719559
High	2000	8.81	0.02730	705	0.719522
	2001	8.51	0.02234	833	0.719043
	2002	10.40	0.02203	1032	0.718237
	2006	13.57	0.02373	1250	0.716778

Table 5
Understory Discrimination Factors 1999-2008

Species		n	r2	p-value	Intercept	error	DF wol	error	DFst	error
Beech	All	30	0.6961	8.16E-75	6889	142	2.40	0.05	2.19	0.05
	Low	10	0.7508	4.67E-23	6326	450	2.20	0.16	2.19	0.14
	Mid	10	0.7305	1.77E-21	5304	239	1.85	0.16	1.68	0.08
	Upper	10	0.9545	1.64E-24	5525	102	1.85	0.02	1.75	0.03
Fir	All	20	0.6791	4.54E-41	6904	379	2.41	0.13	2.19	0.12
	New	12	0.6527	0.0015	7367	554	2.57	0.19	2.34	0.19
	Old	7	0.8425	1.10E-12	5713	437	1.99	0.15	1.81	0.14
Spruce	All	20	0.0134	2.80E-37	-32368	-4835	-11.28	-1.68	-10.27	-1.53
	New	12	0.0053	5.56E-21	-47580	-9836	-16.58	-3.43	-15.10	-3.12
	Old	8	0.0549	2.90E-13	-15976	-4327	-5.57	-1.51	-5.07	-1.37
Sugar Maple	All	19	0.2333	8.96E-39	4954	358	1.73	0.12	1.57	0.11
	Low	10	0.6876	4.04E-21	3555	186	1.24	0.06	1.13	0.06
	Mid	6	0.4341	8.47E-10	3094	454	1.08	0.16	0.98	0.14
	Upper	3	0.3120	0.0027	7424	1410	2.59	0.49	2.36	0.45
Viburnum	All	39	0.7571	1.01E-94	3084	68	1.07	0.02	0.98	0.02
	Low	10	0.6936	2.09E-21	2606	125	0.91	0.04	0.83	0.04
	Mid	9	0.8601	3.35E-19	2437	103	0.85	0.04	0.77	0.03
	Upper	10	0.9232	1.19E-22	2717	86	0.95	0.03	0.86	0.03
	High	10	0.8854	4.94E-05	2569	105	0.90	0.04	0.82	0.03
Wood Fern	All	40	0.8015	5.63E-94	3029	81	1.06	0.03	0.96	0.03
	Low	10	0.7565	3.84E-20	2252	144	0.78	0.05	0.71	0.05
	Mid	10	0.6631	3.78E-20	3240	226	1.13	0.08	1.03	0.07
	Upper	10	0.6717	1.14E-19	3189	258	1.11	0.09	1.01	0.08
	High	10	0.9635	4.90E-07	2439	87	0.85	0.03	0.77	0.03

Table 6
Overstory Discrimination Factors 1999-2006

Species		n	r2	p-value	Intercept	error	DF wol	error	DFst	error
Beech	All	12	0.9389	2.9E-31	5425	170	1.89	0.03	1.72	0.79
	mid	6	0.8643	7.6E-13	6443	107	2.24	0.06	2.04	0.95
	High	6	0.9559	2.5E-13	5451	75	1.90	0.04	1.73	0.80
Spruce	All	6	0.5227	0.1045	-12830	-1539	-4.47	-0.54	-4.07	-0.49
Sugar Maple	All	18	0.0118	0.0118	227	2	1.72	0.07	1.72	0.07
	Low	6	0.9196	0.0025	1869	74	0.65	0.03	0.59	0.02
	Mid	6	0.7842	0.0189	1897	149	0.66	0.05	0.60	0.05
	High	6	0.3629	0.2057	7764	542	2.71	0.19	2.46	0.17
Yellow Birch	All	18	0.8619	2.8E-08	3782	82	1.32	0.03	1.20	0.03
	Low	6	0.5737	0.0811	4221	333	1.47	0.12	1.34	0.11
	Mid	6	0.9282	0.0020	2730	100	0.95	0.03	0.87	0.03
	High	6	0.9745	0.0002	3812	77	1.33	0.03	1.21	0.02

References

Blum J.D., Klaue A., Nezat C.A., Driscoll C.T., Johnson C.E., Siccama T.G., Eagar C., Fahey T.J. and Likens G.E. 2002. Mycorrhizal weathering of apatite as an important calcium source in base-poor forest ecosystems. Nature 417: 729–731.

Blum J.D., Taliaferro E.H., Weisse M.T. and Holmes R.T. 2000. Changes in Sr/Ca, Ba/Ca and Sr-87/Sr-86 ratios between trophic levels in two forest ecosystems in the northeastern USA. Biogeochemistry 49: 87–101.

Dasch, A.A., et al. 2006 The Isotope Geochemistry of Calcium and Strontium in Tree tissues as a Monitor of Calcim Soruces and Cycling in a Base-PoorForested Ecosystem, New Hampshire, USA. A dissertaion submited in partial fulfillment of the requiremetns for the degree of Doctor of Philosophy (Geology) in the University of Michgian.

Dasch A.A., et al. 2006 The Relative Uptake of Ca and Sr into Tree Foliage Using a Whole-Watershed Calcium Addition. Biogeochemistry 80:21-41.

DeHayes, D.H., Schaberg, P.G., Hawley, G.J., Strimbeck, G.R. 1999. Acid Rain Impacts on Calcium Nutrition and Forest Health Alteration of membrane-associatedc alcium leads to membrane destabilization and foliar injury in red spruce. BioScience, Vol. 49, No. 10 pp. 789-800 Published by: American Institute of Biological Sciences

Federer C.A. et al. 1989. Long-term depletion of calcium and other nutrients in eastern U.S. forests. Environ. Manage. 13: 593–601.

Ferguson E.B. and Bollard E.G. 1976. Movement of calcium in woody stems. Ann. Bot. Lond. 40: 1057–1065.

Hogan J.F. and Blum J.D. 2003. Tracing hydrologic flow paths in a small forested watershed using variations in (87)Sr(86)Sr, [Ca]/[Sr], [Ba]/[Sr] and Delta O-18. Water Res. Res. 39.

National Science Foundation. 2010. "The Hubbard Brook Ecosystem Study." Web. http://www.hubbardbrook.org/

Likens G.E., Driscoll C.T. and Buso D.C. 1996. Long-term effects of acid rain: Response and recovery of a forest ecosystem. Science272: 244–246.

Likens G.E. et al. 1998. The Biogeochemsitry of Calcium at Hubbard Brook. Biogeochemsityr 41: 89-173.

Peters, S.C., Blum, J.D., Discroll, C.T.,Likens, G.E. 2004. Dissolution of wollastonite during the experimental manipulation of Hubbard Brook Watershed 1. *Biogeochemistry* 67: 309–329.

WebElements: the Periodic Table on the WWW. The University of Sheffield and WebElements Ltd, UK, 1999. Web. 3 Mar. 2010. <http://www.webelements.com/>.