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Terrestrial Gastropod Responses to an Ecosystem-level Calcium Manipulation in a Northern Hardwood Forest

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ABSTRACT

The effects of acid deposition on soil Ca, and in turn on land snail populations, have been of heightened concern for several decades. We compiled a 10-year record (1997-2006) of gastropod abundance on two small watersheds at the Hubbard Brook Experimental Forest, one of which was treated with a Ca addition in 1999. In years 3-7 post Ca addition, snail abundance in the treated watershed was 73% higher than the reference ($p < 0.001$); there was no significant difference in the three years pre-treatment, and no significant difference in slug abundance in any year. We analyzed relationships between snail density and microsite spatial variation in leaf litter Ca concentration, litter layer thickness, tree species composition, slope, dead wood, and forest floor light levels. We found that snail abundance was significantly correlated with litter Ca concentration ($p < 0.001$), and that abundance was negatively correlated to the importance value of American beech ($p = 0.05$). Isotopic tracer analysis indicated that an average of 76% of calcium in the snail shells 5 years post-treatment was derived from the added Ca. However, interannual variation in snail numbers indicates that there are other factors beyond available Ca with a strong influence on snail abundance.

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INTRODUCTION

Calcium (Ca) is an essential nutrient in plants and animals. Its availability and cycling are governed by numerous factors including atmospheric deposition, soil mineral composition and weathering, plant uptake and growth, forest stand dynamics, and leaching losses (Likens *et al.* 1998; McLaughlin and Wimmer 1999). A decrease in available Ca in the soils of the northeastern US has been thought to result from an increase in acid deposition over the latter half of the 20th century (Driscoll *et al.* 2001; Likens *et al.* 1996), though Hamburg *et al.* (2003) and Yanai *et al.* (1999; 2005) found little evidence for decreased Ca availability in some base-poor northeastern forests.

Since snails require substantial amounts of Ca for reproduction (Crowell 1973; Wareborn 1979), growth (Gomot *et al.* 1989; Ireland 1991), and shell production (Russel-Hunter 1983; Fournié and Chétail 1984) their abundance may serve as a bioindicator of ecosystem-level Ca availability in northern hardwood forests (Hamburg *et al.* 2003). Greater abundance, species richness, and biomass of snails is generally observed on soils rich in Ca compared to soils poor in Ca, (Boycott 1934; Wareborn 1992; Hotopp 2002), while slugs do not generally show similar patterns (Beyer and Saari 1977). Forest stands (Nekola and Smith 1999; Hotopp 2002) and microhabitats (Müller *et al.* 2005) which have higher soil pH (and thus higher Ca availability) also show increased snail abundance relative to sites with more acidic soils.

If depletion of soil Ca leads to a reduction in the concentration of Ca in foliar tissues, the availability of calcium to terrestrial snails, which inhabit the leaf litter, should be reduced (Wareborn 1992; Schaberg *et al.* 2001). This can have ramifications at higher trophic levels; particularly on forest birds which may rely on snails as a source of Ca for producing viable eggs (Graveland *et al.* 1994; Graveland and van der Wal 1996). Because of the critical role snails play in concentrating detrital Ca and making it available at higher trophic levels, they are uniquely suited as a bioindicator of overall Ca availability at the ecosystem level.

Processes other than acid deposition also influence calcium cycling and availability in forest soils. For example, northern hardwood succession results in a shift in the dominant canopy tree species from those with high foliar Ca concentrations, such as pin cherry (*Prunus serotina* L.f.) to those lower in Ca, such as American beech (*Fagus grandifolia* Ehrh.), which in

turn are reflected in forest floor Ca concentrations (Fahey *et al.* 1998). In fact, younger northern hardwood stands appear to have more available Ca in the forest floor and a higher density of gastropods than older stands (Hamburg *et al.* 2003).

In central New Hampshire there has been an increase in the importance of tree species with high foliar Ca concentrations, including sugar maple (*Acer saccharum* Marsh.) and birch (*Betula* spp.), over the past 200 years (Cogbill *et al.* 2002). In addition to available calcium in the forest floor, spatial patterns in gastropod abundance can be influenced by soil moisture, soil pH, soil temperature (and thus indirectly sunlight), presence and quantity of dead wood, slope, microtopography, and the number of predators in the surrounding area (Pilsbry 1948).

Soil moisture has been shown to have an effect on the diversity and abundance of terrestrial snails (Martin and Sommer 2004; Müller *et al.* 2005; Kappes *et al.* 2006; Jubb *et al.* 2006). Snails require relatively high soil moisture levels for survival though there is no direct evidence for a relationship between soil moisture and snail abundance at a microsite scale within a single forest stand. However, Martin and Sommer (2004) did find that forests on wet soils with the same pH as intermediate soils have higher numbers of individuals and species, and Hylander *et al.* (2004) showed that wetter sites had a greater abundance of snails after clearcutting than drier clearcut sites.

The presence of large pieces of dead wood has also been shown to have a positive correlation with gastropod densities and species diversity (Müller *et al.* 2005; Kappes 2005; Kappes 2006; Kappes *et al.* 2006). Dead wood may provide moist microsites in periods when the forest floor is dry (Müller *et al.* 2005), and is often heavily colonized by fungi, which may have the effect of concentrating Ca (Ostrowsky *et al.* 1997). However, dead wood is also a good habitat for salamanders, which are effective snail predators (Hamilton 1932; Burton 1976), so the net effect of large amounts of dead wood on snail abundance may depend on other factors which influence the population dynamics of salamanders and other snail predators. Birds are also known to feed on snails (Betts 1955; Graveland *et al.* 1994). However, birds restrict their predation of snails to periods when they are laying eggs (Graveland and Berends 1997), and chemical data from our study site suggest that small songbirds ingest only two to three snails for each egg produced (Blum *et al.*

2001), so it is unlikely that they have a significant impact on overall snail abundance.

Although many factors affect snail populations in the northern hardwood forest, Wareborn (1992) and Hotopp (2002) suggest that Ca availability is the underlying factor most directly controlling population density. In the study reported here, we explore this question by investigating the determinants of snail community composition and densities in two experimental watersheds at the Hubbard Brook Experimental Forest in central New Hampshire, USA; one with added Ca and one without. While Johannessen and Solhoy (2001) studied the short-term effects of Ca addition on snails, to our knowledge this is the first long-term ecosystem-scale study to address this issue using an experimental manipulation. This approach allows us to directly test the assumption that snail abundance is a reliable metric of overall ecosystem-level Ca availability on the base-poor soils typical of forests in the northeastern United States. We specifically test the following hypotheses:

- 1) Snail abundance at the landscape scale will increase when Ca is added to the forest floor in an ecosystem-scale experimental manipulation. All common snail species will increase in abundance, while slugs will not.
- 2) The increase in snail abundance will be approximately proportional to the increase in the Ca content of leaf litter, and snails will only respond to additional Ca once it is incorporated into the forest floor via leaf litter.
- 3) Forest floor microsites with high litter Ca concentrations, as well as those with shallow slope, thick litter layers, dense canopy cover, and abundant dead wood nearby will have higher snail abundance than sites without these attributes.

MATERIALS AND METHODS

Study site

Snails were collected from Watershed 1 (W1) and west of Watershed 6 (W6) at the Hubbard Brook Experimental Forest (HBEF), a Long-Term Ecological Research site near Woodstock, New Hampshire (43° 56' N, 71° 45' W). These watersheds are approximately 1 km apart on a south-facing slope between 500 and 800 meters elevation. The mean annual precipitation in these watersheds is 140 cm, with an average January temperature of -8°C and an average July temperature of 19°C (Bailey *et al.* 2003).

The bedrock is Rangelly Formation Schist overlain with 1-3m of basal till (Johnson *et al.* 1968; Bormann *et al.* 1970). Soils are predominately spodosols (haplorthods), acid (pH < 4.5) and low in base cations (Gosz *et al.* 1976). The dominant canopy species at the elevation of our study sites are American beech, sugar maple, and yellow birch (*Betula alleghaniensis* Britton). In the late 1800's and the 1910's, the study areas were cut over, primarily for red spruce (*Picea rubens* Sarg.; Bormann *et al.* 1970). In 1938, some of the remaining mature timber was blown down in a hurricane and subsequently salvage harvested. In October 1999, wollastonite (CaSiO₃) was added to W1 at a rate of 1.6 x 10⁶ g Ca/ha to study the effects of increased calcium in a base-poor forest ecosystem with chronic acid deposition (Peters *et al.* 2004). The wollastonite added has a calcium to strontium ratio (Ca/Sr) and strontium isotope ratio (⁸⁷Sr/⁸⁶Sr) different from the parent material at HBEF, making it possible to trace the added Ca as it cycles through the ecosystem (Dasch *et al.* 2006).

The area west of W6 has not been experimentally manipulated, and along with W6 serves as a reference area for many studies at HBEF. This area is an appropriate reference for W1 due to its proximity and similar forest composition, substrate, slope position, and disturbance history (Likens and Bormann 1995). Prior to the Ca treatment in 1999, mean Ca concentrations in the forest floor Oie (L and F) layers on W1 were not significantly different from that on W6, where forest floor chemistry has been monitored systematically since 1976 (Siccama unpublished data). Forest floor Ca concentrations on W6 show no significant long-term trends (Yanai *et al.* 1999). Watershed 6 generally receives slightly more precipitation than does W1 (Bailey *et al.* 2003), though during the June-July period from 1997-2005, the difference was only 3% (Eagar, unpublished data)

Snail collection

Snails were collected once in the summer of 1997 and twice each summer from 1998 thru 2006, using the cardboard sheet method (Hawkins *et al.* 1998; Boag 1982), which produces acceptably representative samples of the gastropod community in northern hardwood forests (Strayer *et al.* 1986). Though individual species likely differ in the efficiency with which they are sampled by this method, community metrics derived from such data estimates are relatively robust (McCoy 1999).

We used recycled brown paperboard, 0.7 mm in thickness, and 0.56 m² in area, held down with small pieces of dead wood. In early June of each year, 15

cardboards were placed in a systematic grid on the forest floor at three elevations (520m, 610m, and 700m) both in W1 and west of W6. The cardboards were placed at a linear spacing of 25m in W1 at each of the three elevations in the treated area.

Cardboards were spaced 5-10m apart west of W6 due to the smaller size of the allotted study area. The location of each cardboard was flagged to allow its reuse each year. Cardboards were randomly placed within a 3m radius of the flag each year, to avoid any direct impact the previous year's cardboard may have had on forest floor characteristics. We avoided placing cardboards anywhere without an intact litter layer (rock outcrops, streambeds, washouts, and patches of moss), as well as in small depressions that might collect water and result in flooded cardboards at the time of collection. Fresh cardboards were used each year, and all cardboards were removed immediately after the August collection.

Snails were collected from the underside of each cardboard in early July and early August, one to two days after a >7 mm rain event, so that the cardboards were still moist but not saturated at the time of collection. Only snails adhering directly to the cardboard were collected; those in leaf litter stuck to the cardboard were not collected due to the highly variable amount of litter adhering to each cardboard. Slugs were counted, but not collected. The snails were drowned in distilled water and then preserved in 95% ethanol. Each year two crews of two to three people received standardized training by Steven Hamburg before their first collection to reduce collecting bias. The crews collected snails from the different elevational transects in the same order on both watersheds and the crews alternated the watersheds they collected from between the two collections.

All snails from collection years 1997 to 2005 were identified to genus or species, using Burch (1962) as a taxonomic reference. Of the 6500+ snails collected in these years, approximately 10% were too immature to be keyed to species with confidence, and 6% were lost or damaged in collection or transport and could not be keyed to species with confidence (Table 1).

Calcium tracer analysis

The wollastonite (CaSiO_3) that was added to W1 has Ca/Sr and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios that are distinct from the background values in the watershed (Dasch *et al.* 2006) and thus can be used as a tracer of Ca and Sr derived from CaSiO_3 dissolution that has been directly or indirectly incorporated into snail biomass.

Since $^{87}\text{Sr}/^{86}\text{Sr}$ ratios cannot be modified by biological uptake (Blum *et al.* 2000), it is possible to calculate the proportion of Sr, and thus Ca, from either source in any snail.

Blum *et al.* (2000) found that there was an increase in the Ca/Sr ratio of caterpillars and snails compared to understory foliage in an untreated watershed at Hubbard Brook. The pre-treatment (1999) data from this study allow a determination of the Ca/Sr discrimination factor (DF; $(\text{Ca}/\text{Sr}_{\text{organism}})/(\text{Ca}/\text{Sr}_{\text{nutrient source}})$) for snails, which can then be used to determine the proportion of Ca in the post-treatment (2005) snails that is derived from the added wollastonite (Dasch *et al.* 2006).

We measured Ca/Sr and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of *Discus catskillensis* (Pilsbry, 1896) from six randomly chosen cardboards in W1 (2 per elevation) from 2005 and 1999. Whole snails were dried at 60°, weighed, and pulverized using virgin vials and balls in a Wig-L-Bug amalgamator. The samples were digested in ultrapure 5% nitric acid, evaporated to dryness and diluted to appropriate concentration ranges. An aliquot of each sample was analyzed for Ca and Sr concentrations using inductively coupled plasma optical emission spectrometry (ICP-OES) calibrated to a standard curve. Analyses of secondary standards agreed within 5% of certified values. Another aliquot of each sample was eluted through ion-specific quartz cation exchange columns using Sr-Spec resin. Isotope ratios were measured with a Finnigan MAT 262 thermal ionization mass spectrometer to determine the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios after correction for instrumental mass bias following methods detailed in Blum *et al.* (2000).

Microsite analysis

In 2005, each of the 90 sampling microsites (15 replicates x 3 elevations x 2 watersheds) was characterized with respect to tree species composition, microtopography, leaf litter composition, dead wood, leaf litter Ca content, and sunlight reaching the forest floor. All trees with a diameter at breast height (DBH) > 2cm were measured in a 3m radius surrounding each cardboard, and all trees with a DBH > 10 cm were characterized within a 5m radius. Tree species abundance is expressed as a relative importance value, which is the mean of the fraction of total basal area and the fraction of total stem density represented by each species. Slope was measured from a point five meters downslope to a point five meters upslope of the cardboard.

Leaf litter (O_i) was collected from three points 1.5 m to the north, west, and south of each cardboard using a circular template 20cm in diameter. If any sampling point landed on a rock a sample was taken to the east of the designated location. Composite litter samples were sorted to species, dried at 60°C, and weighed. For chemical analysis, the samples from three cardboard sites from each elevation within each watershed were chosen with a stratified random method in order to cover the full length of the transect at each elevation. These samples were milled, homogenized, and subsampled. Subsamples (0.2g) of the three most common species (sugar maple, beech, and yellow birch) were digested for 15 minutes at 190°C using 70% nitric acid in a microwave digestion system (ETHOS 1600), and analyzed for Ca concentrations using ICP-OES. Accuracy of the Ca measurements was assessed using peach and pine references, which yielded values 12% (1.3 times the certified confidence interval) and 4% (within the certified confidence interval) greater than their certified values, respectively. A total leaf litter Ca concentration for each cardboard was calculated using sorted species masses and the mean Ca concentration for each species at that elevation on that watershed. Errors were propagated through this calculation. At five cardboard locations, less than 85% of total leaf litter mass was composed of the three species we analyzed; these locations were excluded from the Ca concentration analysis.

The volume of all dead wood within 2m of each cardboard was measured using four diameter classes (2-10cm, 10cm-20cm, >20cm), and length. Approximate total dead wood volume within this radius was calculated using approximate mean diameters for each class (6cm, 15cm, and 25cm).

Hemispherical photos were taken above the site of each cardboard on 9 September 2005 and analyzed for direct site factor in June and July, using Gap Light Analyzer 2.0 software (Frazer 1999). The resulting data were used to estimate the percentage of time each cardboard location receives direct sunlight.

Statistics

In order to reduce stochastic effects of collection day conditions on snail abundance, we expressed snail abundance as the mean snail count per collection per square meter of cardboard for each year in all subsequent analyses. Snail and slug abundances were compared between the reference and treatment watersheds for each collection year using a two-tailed unpaired t-test. This analysis was conducted for whole watersheds ($n = 45$ per

watershed) as well as for each elevation band ($n = 15$ per watershed per elevation). We conducted similar analyses for the three most common snail species, which together accounted for 90% of all snails keyed to species (Table 1). Site-level snail species richness in 2005 (for each $n = 6$ transects, watershed x elevation) was calculated using jackknifed estimation algorithm (Hines 1996), as recommended by McCoy (1999).

In order to remove interannual variability, treatment effect was assessed by comparing the differences observed in the three years pre-treatment (1997-1999) to years 3-7 post-treatment (2002-2006). We ran an ANOVA on all snail density data from the pre-treatment period with watershed, elevation, and year as factors. We repeated the analysis for the post-treatment years 2002-2006.

We implemented a General Linear Mixed Modeling procedure using Restricted Maximum Likelihood (on $n = 85$ cardboards) to determine fixed effects of seven variables measured at each cardboard microsite (elevation (m), slope (%), direct transmittance of the canopy (%), total volume of dead wood within 2 meters (cm^3/m^2), O_i (litter) layer mass, O_i layer Ca concentration (mg/g), and the relative importance value of beech (%) as measured in 2005) on observed snail abundance, slug abundance, and the abundance of the three most common species in 2005.

RESULTS

In all, 7214 snails were collected, and 1737 slugs were observed, for an average of 7.6 snails and 1.8 slugs per square meter of cardboard per collection. The ratio of slugs to snails (0.24) is much lower than that observed by Gleich and Gilbert (1976) using similar methods in hardwood forests in Maine (0.64 slugs per snail). The most common snail species collected were *Discus catskillensis*, *Striatura exigua* (Stimpson 1850), and *Zonitoides arboreus* (Say 1816); these species accounted for 70% of snails found by Gleich and Gilbert (1976) across diverse habitats in central Maine, and are all native species reported as common in New Hampshire by Baker (1942). We did not systematically identify slugs during snail collections, but noted that non-native *Arion* species were common.

Snail density trends

Snail abundance in W1 steadily increased between the 1999 application of wollastonite and its peak in 2004, and subsequently decreased (Figure 1a). Snail abundance west of W6 shows a similar pattern,

though with a much lower and less distinct peak value in 2003. Snail densities in W1 (treated) relative to west of W6 (reference) is significantly higher and far more variable since 2002, two years after the addition of Ca (Figure 1b), indicating that the Ca addition has increased snail abundance though considerable interannual variability exists, and there was approximately a two year lag between the Ca treatment and an observable increase in snail abundance. The years 3-7 following the Ca addition show 71% greater snail abundance in the treated watershed compared to the reference (two-tailed heteroscedastic t-test on 4-year cardboard-level means; $DF = 72$; $t = 3.48$; $p < 0.001$); the difference in the three years preceding the treatment was only 7% ($DF = 72$; $t = 0.170$; $p = 0.86$; Figure 1b). Slug abundance showed no trends over time, and no significant difference between the treatment and the reference in any year, (Figure 2).

An ANOVA run on the three pre-treatment years ($n = 90$ cardboards) shows that snail abundance was significantly affected by elevation ($p < 0.0001$) and year ($p = 0.001$), but not by watershed ($p = 0.80$). In years 3-7 post-treatment, all three factors (elevation, year, and watershed) were significant determinants of snail abundance ($p < 0.0001$ for all factors).

Snail abundance was affected by the Ca treatment in two of the three elevation bands studied. Differences in snail abundance between watersheds have increased in significance post-application at the middle and upper elevations, but not at the lower elevation (Figure 3). In the upper elevation sites, the ratio of snails in the treated watershed to the untreated watershed went from 1.8 pre-treatment ($DF = 23$; $t = 1.96$; $p = 0.06$) to 2.4 post-treatment ($DF = 25$; $t = 2.58$; $p = 0.02$). In mid-elevation sites, where snail abundance has been consistently low over all 10 years of the record (Figure 3b), the ratio shifted from 3.4 ($DF = 17$; $t = 2.80$; $p = 0.01$) to 4.0 ($DF = 16$; $t = 6.42$; $p < 0.0001$). In the low elevation sites, the ratio shifted from a significant 0.7 pre-treatment ($DF = 18$; $t = -2.84$; $p = 0.01$) to a non-significant 1.3 post-treatment ($DF = 28$; $t = 0.29$; $p = 0.77$).

Discus catskillensis was significantly more abundant in the treated watershed than in the reference area in years 3-6 post-treatment (t-test on annual data, $DF = 6$, $t = -2.61$; $p = 0.04$) as was *Striatura exigua* ($DF = 5$; $t = -2.68$; $p = 0.04$), while *Zonitodes arboreus* showed no significant treatment effect (Figure 4). Pre-application abundances show no significant differences between the watersheds for any of the

three species. *Discus catskillensis* and *Striatura exigua* were present in approximately equal numbers both before and after the treatment in both watersheds. *Zonitodes arboreus* accounted for 24% of snails collected pre-treatment on W1, but only 8% post treatment, as it did not respond to the treatment with increased abundance.

Calcium tracer analysis

The average $^{87}\text{Sr}/^{86}\text{Sr}$ ratio in snails on W1 shifted from 0.72020 in 1999 to 0.71695 in 2005, meaning that on average of 23% of the Sr in the 2005 snails was derived from wollastonite. To determine the DF for snails we consider the Ca/Sr and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the 1999 snails and the 1999 forest foliage (Blum *et al.* 2000). Foliage $^{87}\text{Sr}/^{86}\text{Sr}$ ratios data for 1999 (Blum *et al.* 2002) and those for W1 snails from the same year are plotted in Figure 5a as Ca/Sr versus $^{87}\text{Sr}/^{86}\text{Sr}$; mixtures between endmember chemical reservoirs plot as lines on this diagram (see Dasch *et al.*, 2006). Note that the mean $^{87}\text{Sr}/^{86}\text{Sr}$ for snails and foliage are nearly identical (0.72020 vs 0.72011), but that the variability of the snails is greater than the foliage. This results from the fact that snails ingest organic matter from a relatively small area of the forest and thus sample the heterogeneity of the forest $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, whereas composite foliage samples average out much of the heterogeneity in the canopy.

The much higher Ca/Sr ratios of snails collected in 1999 (pre-treatment) compared to foliage collected in the same year is the result of the preferential incorporation of Ca over Sr into snail shells and the heterogeneity of Ca/Sr ratios in the forest foliage (Dasch *et al.* 2006). A range of DFs between 3 and 4 is required to explain the 1999 snail Ca/Sr ratios if we assume that their nutrient source is forest foliage. This is shown diagrammatically on Figure 5a where the Ca/Sr concentrations for each snail shell analyzed (raw data shown as asterisks) are divided by 3 and by 4 and plotted on the diagram (as + signs connected by a bold line); this brings the resulting Ca/Sr ratios within the range of Ca/Sr for foliage. This range is similar to that reported by Odum (1951) in cultured mollusks from a range of aquatic habitats, and to that found in freshwater snails by Likins *et al.* (1963) across a wide range of experimentally manipulated Ca/Sr ratios ($DF = 3.1$ - 3.7 for $\text{Ca/Sr} = 3.1 - 11$).

The finding of a DF of 3-4 can be tested using the 2005 post-application snail analyses. If snails did not discriminate between Ca and Sr ($DF=1$), as is the case for wood ferns (Dasch *et al.* 2006), then the 2005 snails would plot on a mixing line between foliage

and the Ca/Sr and $^{87}\text{Sr}/^{86}\text{Sr}$ of the wollastonite that was added to the forest. If the DF is between 3 and 4, then the 2005 snails would be expected to plot on a mixing line connecting foliage with a Ca/Sr that is between 3 and 4 times the value of the wollastonite, and with a $^{87}\text{Sr}/^{86}\text{Sr}$ ratio that is identical to the wollastonite; which is exactly what is observed (Figure 5b).

Based on the finding of a snail DF between 3 and 4 we can calculate the proportion of Ca in the 2005 snails that is derived from wollastonite following the methodology and mixing equations presented in Dasch *et al.* (2006). We calculated high and low bounding estimates of the proportion of wollastonite Ca assuming a DF of 3 and 4, and estimated that the mean fraction of wollastonite-derived Ca in snails collected on W1 in 2005 as 73-79%. The range for individual snails varies between 59 and 92%.

Site factors contributing to snail abundance

The tree species composition of the forest appears to be an important determinant of snail abundance. A cardboard-level regression of snail abundance in 2005 against the relative importance value of American beech within 5m of the cardboard shows a highly significant negative trend ($n = 90$; $p < 0.001$), though there is a great deal of residual variance remaining in the data ($R^2 = 0.12$). Looking at each watershed individually, W1 shows no significant relationship (Figure 6 dashed line; $p = 0.23$; $R^2 = 0.03$), but W6 shows a highly significant relationship that explains substantially more of the variance (Figure 6 solid line; $p < 0.001$; $R^2 = 0.30$). On both watersheds, the relative importance value of beech is greatest in the upper and middle elevation bands, (Figure 6) and least in the lower elevation band, which has greater densities of sugar maple, yellow birch, and white ash (*Fraxinus americana* L.).

Mean leaf litter Ca concentration values for W1 were higher than W6 for all three tree species tested: beech (12.32 vs. 7.97 mg/g; DF = 11; $t = 4.08$; $p = 0.002$), sugar maple (20.20 vs. 8.72 mg/g; DF = 9; $t = 4.41$; $p = 0.002$), and yellow birch (22.36 vs. 10.88 mg/g; DF = 11; $t = 4.67$; $p < 0.001$). When separated by elevation, the upper elevation in W1 had the highest Ca concentration values for all three species (beech = 14.9 mg/g; sugar maple = 21.9 mg/g; yellow birch = 26.8 mg/g). W6 shows its highest values at its lowest elevation (beech = 9.3 mg/g; sugar maple = 9.6 mg/g; yellow birch = 14.3 mg/g).

Leaf litter Ca concentration data, when separated by elevation, show a strong linear correlation with the

2005 snail density data ($n = 6$; $R^2 = 0.93$; $p = 0.002$; Figure 7a). Snail species richness per cardboard for 2005 when similarly separated by elevation shows non-significant but suggestive relationship with leaf litter Ca concentrations ($n = 6$; $R^2 = 0.43$; $p = 0.16$; Figure 7b). Figure 7a also shows that regardless of treatment, the Ca concentration of the leaf litter is an important factor regulating snail abundance and species richness. The lower elevation W6 Ca concentration values which are comparable to the middle elevation WS1 values result in snail densities that fall within a similar range.

A General Linear Mixed Model run on cardboard-level abundances of snails and slugs in 2005 shows that the Ca concentration of the litter layer was by far the most important factor controlling total snail abundance, as well as the abundance of *Discus catskillensis* and *Striatura exigua* in our study area (Table 2). Slugs responded only to elevation, with greater numbers at higher elevations. The relative importance value of beech at the cardboard scale showed significant negative effects on total snail abundance, and the abundance of *Zonitoides arboreus*. Elevation also had a negative effect on the abundance of *Zonitoides arboreus*. None of the dependent variables in Table 2 appeared to be significantly affected by slope, canopy transmittance, the volume of dead wood within 2m, or total litter layer mass.

DISCUSSION

Snail abundance and calcium

Snail abundances on both W1 and W6 have increased since 1997, though at rates which differ by watershed (Figure 1) and elevation (Figure 3). The ratios for the upper and middle elevation snail populations appear to have been more affected by the added Ca than the lower elevation. The overall increase in snail populations in W1 can be linked to the additional available Ca in the soil and in the leaf litter (Figure 7). The lack of a similar change in slug abundance (Figure 2; Table 1) suggests that the increase in snails is the direct effect of the availability of Ca, which is required by snails (Fournié and Chétil 1984) in proportionally larger amounts than by slugs. If some other, indirect effect of the Ca addition were responsible (increased pH or changes in forest productivity), we would expect a similar effect on slugs, which are physiologically and ecologically similar to snails aside from lower dietary Ca requirements. Other potential differences between snails and slugs (*e.g.* size, visibility, and

palatability to predators) fail to satisfactorily explain the observed between-watershed difference in snail abundance (Figure 1).

The fact that snail populations increased post-treatment on both watersheds, and that populations on both the treated and untreated watersheds have declined since 2004 (Figure 1a), suggests that available Ca is not the only factor affecting snail abundance. Our data suggest that snail population size is also affected by local vegetation composition, and available Ca in the litter layer, neither of which have changed appreciably on the reference watershed at the time scale of the last 10 years (Siccama, unpublished data). The increase in snail abundance on the reference watershed (Figures 1a, 3, and 4) is therefore difficult to explain, and highlights the paramount importance of an untreated reference in this type of study. Without the reference watershed, we would be led to conclude that the treatment effect is much stronger than in fact it probably is.

The presence of American beech negatively influences snail abundance, while the presence of sugar maple, a calciphilic species (Likens and Bormann 1970) does not seem to correlate with snail density. The lower Ca concentration of American beech litter may make this species a poor food source for terrestrial snails and thus may be detrimental to their growth and reproduction. However, snail abundance does not correlate with the microsite importance value of sugar maple (analysis not shown). This is contrary to the results of Hotopp (2002) who found that sugar maple abundance is positively correlated with snail density. However, our analysis of leaf litter shows that sugar maple and yellow birch do not have significantly different leaf litter Ca concentrations at five out of six elevation transects, so if snails respond to litter Ca concentration, these species should have nearly equal effects. In terms of composition, then, this effect will be most evident as a negative effect of beech, which is the only major species at our sites with significantly lower litter Ca concentrations (based on our analyses as well as those of Likens and Bormann 1970; Fahey *et al.* 1988; Dasch *et al.* 2006). Analysis of the 2005 data using General Linear Mixed Modeling shows that high relative importance values of American beech negatively affect total snail abundance independent of Ca concentration (Table 2), which is unexpected if the effect of beech abundance on snails is mediated entirely through litter Ca concentration. We cannot rule out that there may be other, indirect effects of beech on

snails, though how those effects might be mediated remains unclear.

Insights from tracer analysis

The finding that snails have a Ca/Sr discrimination factor between 3 and 4 is generally consistent with other studies of biogenic CaCO₃ which, unlike vegetation, is known to strongly incorporate Ca preferentially over other alkaline earth elements (Chivas *et al.* 1986). The finding that the snails in the treated watershed received between 59 and 92% (mean=76%) of their total Ca intake from the added tracer is also consistent with recent studies of vegetation in the same watershed. On average, tracer Ca in foliage from wood ferns (*Dryopteris spinulosa* Marsh.) and *Viburnum alnifolia* (Marsh.), an understory shrub species, increased every year from 2000 to 2004 and foliage collected in 2004 had derived 50-81% of its Ca from added wollastonite (Dasch *et al.* 2006). Foliage from sugar maple and yellow birch increased in tracer Ca each year between 2000 and 2003 and in 2003 had derived 11-40% of its Ca from added wollastonite, with 32-48% at mid and high elevation within the watershed (Dasch *et al.* 2006). Although we do not have data on the amount of wollastonite-derived Ca in the forest floor or tree foliage in 2004, it is reasonable to assume that, like wood fern and *Viburnum*, it continued to increase from 2003 values. Is it also possible that a portion of the tracer Ca found in snail shells could be the result of direct uptake of Ca from particulate wollastonite; snails are known to feed directly on mineral Ca (the shells of dead snails) in some instances (Cadee 1999). However, the two-year lag in response of snail abundance (Figure 1) is consistent with the hypothesis that snails had access to added Ca only after it was incorporated into the leaf litter.

This lag may be attributable to a number of processes. Leaf litter in the treated watershed had a low proportion of wollastonite-derived Ca (10% or less) in the first two growing seasons post-treatment (Dasch *et al.* 2006), so a diet comprised exclusively of leaf litter would not yet have been substantially enriched in Ca. Even by 2002 when new leaf litter was substantially enriched in Ca, much of it wollastonite-derived, it would have been mixed with older, less-enriched litter in the Oe layer in a snail's diet. Some additional lag in whole-snail isotopic signatures may be attributable to a snail's integration of the isotopic signature of its diet over multiple years of shell growth. However, this effect may be limited, as snails of similar size to those most

abundant in our collections may need less than a year to mature (Baur 1989).

Other microsite factors

Leaf litter calcium concentrations, while strongly positively correlated with snail abundance, are certainly not the only factor influencing snail abundance. While cardboards with the highest snail densities do have relatively high leaf litter calcium concentrations, there were also cardboards with high leaf litter calcium concentrations and low snail densities. High litter calcium concentration is a requirement for high snail abundances but it is not sufficient; other factors must limit the abundance of snails at these cardboards. The relationship between leaf litter calcium concentrations and snail abundance (Figure 7a) were very similar across the two watersheds with the lower levels on W1 similar to those found west of W6 with comparable snail densities, suggesting that leaf litter Ca does have some influence on snail abundance, as suggested by Hotopp (2002).

The slope of a site, which is one factor controlling soil thickness and moisture, was not strongly related to snail densities. Sunlight (measured as direct transmittance) is another factor that might be expected to influence snail abundance by altering soil moisture and temperature. Little research has been done on the effects of canopy transmittance on gastropod abundance, but Lange (2003) did find a positive correlation between snail species richness and percentage canopy cover in tropical systems. We found no such relationship at Hubbard Brook in 2005. However, since the sites we studied had a small range of canopy transmittance values in 2005 (leaf area index on these watersheds generally ranges from 5-7; Rhoads *et al.* 2002; 2004), this result probably cannot be generalized to other systems. We saw no evidence that the 1998 ice storm had an impact on snail abundance in our study area, despite its opening up the canopy and allowing more light to reach the forest floor (Rhoads *et al.* 2002). It seems probable that in moist climates, only very dramatic changes in canopy openness have a strong effect. For example, Hylander *et al.* (2004) found that in recent clearcuts, soil moisture is a very important factor controlling the size of the surviving snail populations, and in unfertilized, annually mowed fields near our study site we have observed the almost complete absence of snails (unpublished data).

Müller *et al.* (2005) reported a positive relationship between dead wood, snail abundance, and species richness, and hypothesized that the effect was due

to the moderating effect of dead wood on seasonal moisture variability, as well as higher pH and Ca content in microsites under dead wood. The dead wood data we took in 2005 did not show a positive correlation with the observed abundance of snails or slugs. Given natural variance in deadwood distributions, far greater statistical power would be required to determine whether dead wood of particular species, diameters, or decay classes had an effect on gastropod abundance.

Seasonal and temporal variability of snail populations may in part explain the observed interannual variance in snail density. Longitudinal studies of local snail and slug populations (*e.g.* Strandine 1941; Hunter 1966; Gleich and Gilbert 1976; Kralka 1986) show considerable seasonal cyclicity, which varies greatly by species. However, results from Kralka (1986) suggest that highly suitable sites tend to have greater snail abundance than less suitable sites throughout the growing season, and Gleich and Gilbert (1976) found that gastropod abundance in forested sites in Maine is relatively stable between July and August, so our twice-annual sampling is probably sufficient to capture broad-scale interannual changes in snail populations among sites of varying quality.

Other untested variables (pH, soil moisture, populations of predators, herbaceous cover) may also influence snail abundance at various spatial scales. Snails exist as part of a complex and diverse forest floor faunal community with multiple trophic levels (Burton and Likens 1975), and other components of this community have been shown to respond to the Ca addition on W1 (Fisk *et al.* 2005). It is likely that no single factor determines snail abundance, but rather a combination of many complex, interacting factors. For example, beech bark disease has had the long-term impact of increasing the importance of beech in the forest understory at HBEF (Hane 2003), an effect which has been accelerated by the 1998 ice storm (Rhoads *et al.* 2002). Therefore, the results of this study, which show a negative correlation with American beech (Figure 6), suggest that the increasing importance of beech may negatively impact snail abundance over time.

Our data show that two of the most common snail species in our collections (*Discus catskillensis* and *Striatura exigua*) responded positively to Ca addition, while another species (*Zonitoides arboreus*) was unaffected, so that its abundance relative to the total snail population decreased with the Ca treatment. Interestingly, Boycott (1934) concluded

that a European congener of this species was a calcifuge, meaning its abundance was negatively correlated with soil Ca. It is possible that the *Zonitoides* species compete effectively under low Ca availability, but poorly when Ca availability is high. Indeed, the tolerance of this species for acidic environments appears to be high; Baker (1942) reports finding *Zonitoides arboreus* even under the bark of old pine logs. However, even this species showed a weak negative correlation with the importance value of beech, once again hinting that litter Ca content is not the only way in which canopy species may affect snail populations (Table 2). Unfortunately, our ability to draw conclusions about why individual species responded differently to the treatment is quite limited, as little is known about the autecology of these terrestrial snail species.

Conclusions

We confirmed our hypothesis that snail abundance at the landscape scale increases when Ca is added to the ecosystem, suggesting that snail abundance is a reliable indicator of overall Ca availability at the ecosystem level. Two of the three most common snail species (*Discus catskillensis* and *Striatura exigua*) increased significantly in abundance, but *Zonitoides arboreus* did not. Analysis of the isotope tracer indicates that by 2005, the sixth growing season post-treatment, snails in the treated watershed had derived on average 76% of their Ca from the added wollastonite. Slugs showed no increase in abundance after the Ca treatment, supporting the hypothesis that snails are limited by Ca availability at Hubbard Brook. Previous studies have suggested that Ca availability is a very important driver affecting snail densities (Wareborn 1992; Hotopp 2002). There has recently been much concern about a decrease in plant-available Ca due to acidic deposition (Driscoll *et al.* 2001), which might also have a direct effect on snail abundance (Schaberg *et al.* 2001). This study suggests that at HBEF, Ca availability is the most important factor limiting snail populations (Figure 7; Table 2), though to what extent observed trends in plant-available Ca are significant and attributable to acidic deposition is debated (Yanai *et al.* 1999, 2005; Hamburg *et al.* 2003). We have presented the beginning of a long-term data set tracking snail abundance in an intensively studied ecosystem-level manipulation, which over time we expect to dramatically improve our understanding of the ecosystem processes regulating Ca and community dynamics in northern hardwood forests.

Of the microsite variables we examined, only litter Ca concentration strongly explained the variability in snail abundance. The importance value of beech in the immediate vicinity of the cardboard appeared to have a weak independent effect on total snail abundance, as did elevation. The results of our GLMM show that despite some highly significant relationships, our ability to explain the total variance in snail abundance at the microsite level is still quite poor. Despite these limitations, we were able to better explain drivers of snail abundance at the ecosystem and microsite spatial scales. Further study is needed to understand the factors influencing what currently appears as the stochastic residual variance in gastropod populations.

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Table 1. Number of snails identified by taxa (genus or species) from W1 (which was received a Ca addition in October 1999) and west of W6 (reference) in the Hubbard Brook Experimental Forest over a nine year period (1997-2005), along with total counts for slugs and snails. Note that in 1997, there was only one collection date, while in all other years there were two. Snails collected in 2006 were not identified, only total counts were made. Immature snails were those that did not show distinguishing shell characteristics under 10x magnification.

W1 (Ca treatment in 1999)	1997	1998	1999	2000	2001	2002	2003	2004	2005
<i>Striatura exigua</i> (Stipmson, 1850)	43	51	50	21	209	194	241	307	249
<i>Discus catskillensis</i> (Pillsbry, 1896)	79	98	57	55	95	208	141	253	219
<i>Zonitoides arboreus</i> (Say, 1816)	23	17	95	13	55	35	33	68	81
<i>Succineidae</i>	11	6	5	10	18	19	22	29	12
<i>Helicodiscus cf. shimeki</i> (Hubricht, 1962)	3	7	19	16	3	2	6	13	6
<i>Euconulus cf. polygyratus</i> (Pillsbry, 1899)	0	0	0	1	2	4	22	82	4
<i>Vertigo</i>	0	0	0	0	1	0	20	9	3
<i>Stenotrema fraternum</i> (Say 1816)	0	0	0	0	0	0	0	1	1
<i>Triodopsis albolabris</i> (Say 1816)	0	0	0	0	1	0	0	0	1
<i>Triodopsis tridentate</i> (Say 1816)	0	0	0	0	0	2	0	1	1
<i>Appalachiana sayana</i> (Pillsbry 1906)	0	0	0	0	0	0	0	0	0
<i>Anguispira alternata</i> (Say 1816)	0	0	0	0	1	0	0	0	0
<i>Zoogenetes harpa</i> (Say 1816)	1	0	0	0	0	0	0	0	0
<i>Cionella</i>	0	0	0	0	1	0	0	0	0
<i>Strobilops</i>	0	0	0	0	0	0	0	1	0
Immature	12	15	21	2	31	57	144	67	61
Unknown	10	7	21	12	16	36	23	103	41
Total snails	182	201	268	130	433	557	652	934	679
Total slugs (not keyed)	67	97	150	59	135	81	47	94	94

West of W6 (reference)	1997	1998	1999	2000	2001	2002	2003	2004	2005
<i>Striatura exigua</i> (Stipmson, 1850)	0	51	31	35	156	88	215	173	71
<i>Discus catskillensis</i> (Pillsbry, 1896)	32	133	63	63	126	177	119	124	83
<i>Zonitoides arboreus</i> (Say, 1816)	12	14	42	11	51	21	33	58	61
<i>Succineidae</i>	11	5	7	3	8	33	22	35	10
<i>Helicodiscus cf. shimeki</i> (Hubricht, 1962)	0	1	14	3	4	0	5	11	8
<i>Euconulus cf. polygyratus</i> (Pillsbry, 1899)	0	1	0	0	0	0	1	0	0
<i>Vertigo</i>	0	0	0	0	0	0	1	1	0
<i>Stenotrema fraternum</i> (Say 1816)	0	0	0	0	0	5	1	1	0
<i>Triodopsis albolabris</i> (Say 1816)	0	0	1	1	2	1	0	1	0
<i>Triodopsis tridentate</i> (Say 1816)	0	1	0	0	0	0	0	1	0
<i>Appalachiana sayana</i> (Pillsbry 1906)	0	0	0	0	0	0	0	2	1
<i>Anguispira alternata</i> (Say 1816)	0	0	1	0	0	0	0	0	0
<i>Zoogenetes harpa</i> (Say 1816)	0	0	0	0	0	0	0	0	0
<i>Cionella</i>	0	0	0	0	0	0	0	0	0
<i>Strobilops</i>	0	0	0	0	0	0	0	0	0
Immature	0	23	16	0	23	9	103	53	31
Unknown	0	14	10	6	26	19	23	42	9
Total snails	55	249	185	122	396	353	523	502	274
Total slugs (not keyed)	30	61	96	71	176	82	79	70	105

Table 2. Coefficients and p -values of trends in a multiple regression of each dependent gastropod abundance variable (columns) against each input microsite variable (rows). Data are shown only for fixed effects determined to be significant at the $\alpha=0.10$ level.

	Total snails	Total slugs	<i>Discus catskillensis</i>	<i>Striatura exigua</i>	<i>Zonitoides arboreus</i>
Elevation (m)		+ 0.012 ($p=0.003$)			- 0.009 ($p=0.020$)
Slope (%)					
Canopy Transmittance (%)					
Dead wood volume (cm ³ /m ²)					
Oi layer mass (g/m ²)					
Oi Ca concentration (ppm)	+ 0.805 ($p=0.0003$)		+ 0.293 ($p=0.0003$)	+ 0.293 ($p=0.011$)	
Beech importance value (%)	- 0.059 ($p=0.050$)				- 0.020 ($p=0.011$)

Figure 1. a) Mean snail densities from W1 (which received a wollastonite (CaSiO_3) addition in October 1999, indicated by an arrow) and west of W6 (reference) in the Hubbard Brook Experimental Forest over a ten year period (1997-2006). Error bars are 95% confidence intervals. **b)** Ratio of snail density on treated watershed (W1) to the reference (west of W6). The dashed line is the mean ratio for the three years preceding the treatment.

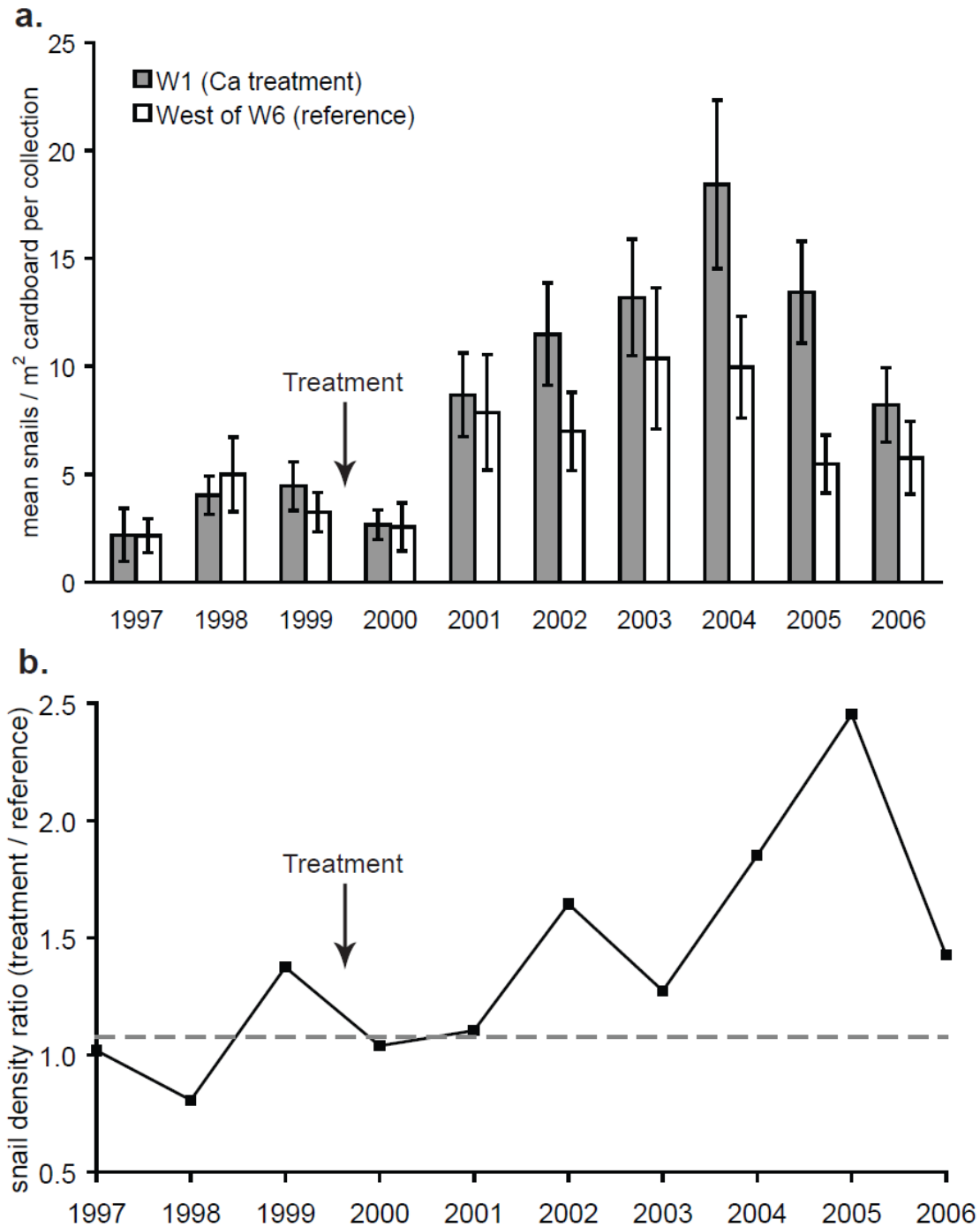


Figure 2. Mean slug densities in W1 (which received a Ca addition in October 1999 indicated by an arrow) and west of W6 (reference) in the Hubbard Brook Experimental Forest from 1997-2006.

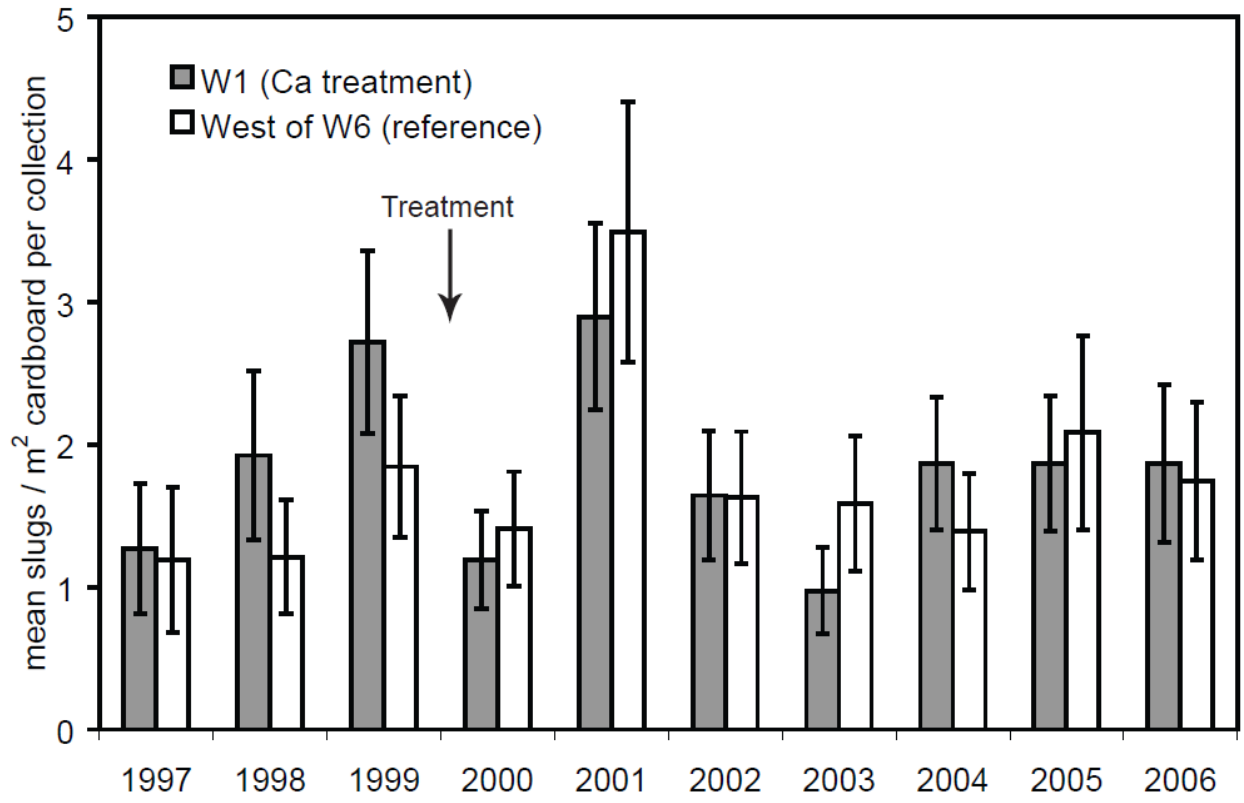


Figure 3. Snail densities at three elevations in W1 (which received a Ca addition in October 1999 indicated by an arrow) and west of W6 (reference) in the Hubbard Brook Experimental Forest over a ten year period (1997-2006). Error bars are 95% confidence intervals.

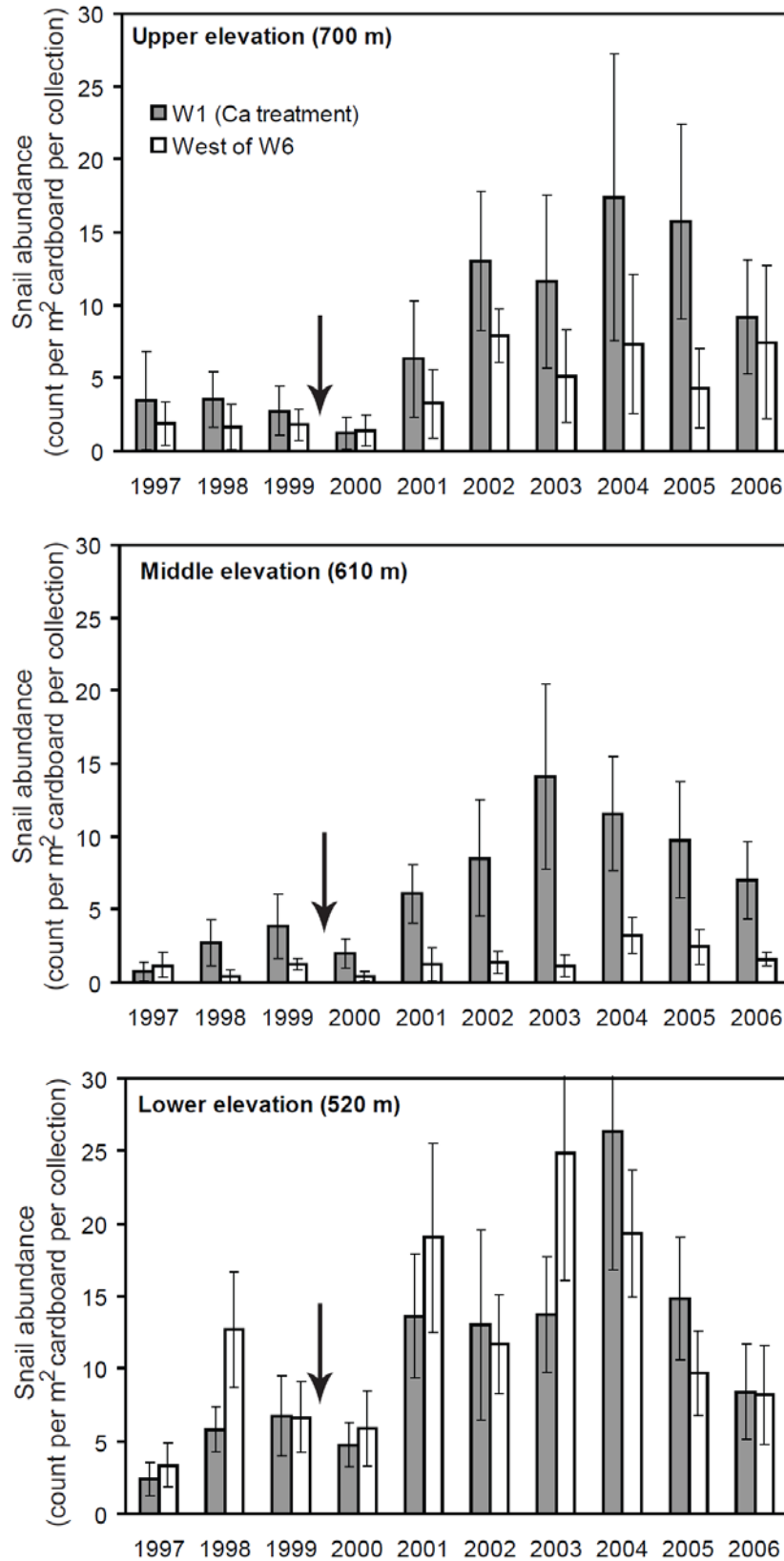


Figure 4: Density of snails by watershed by species in W1 (which received a Ca addition in October 1999 indicated by an arrow) and west of W6 (reference) in the Hubbard Brook Experimental Forest from 1997-2005. Only the three most frequently collected species are shown; together these account for 75% of all snails collected and 90% of all snails keyed to species. Error bars are 95% confidence intervals.

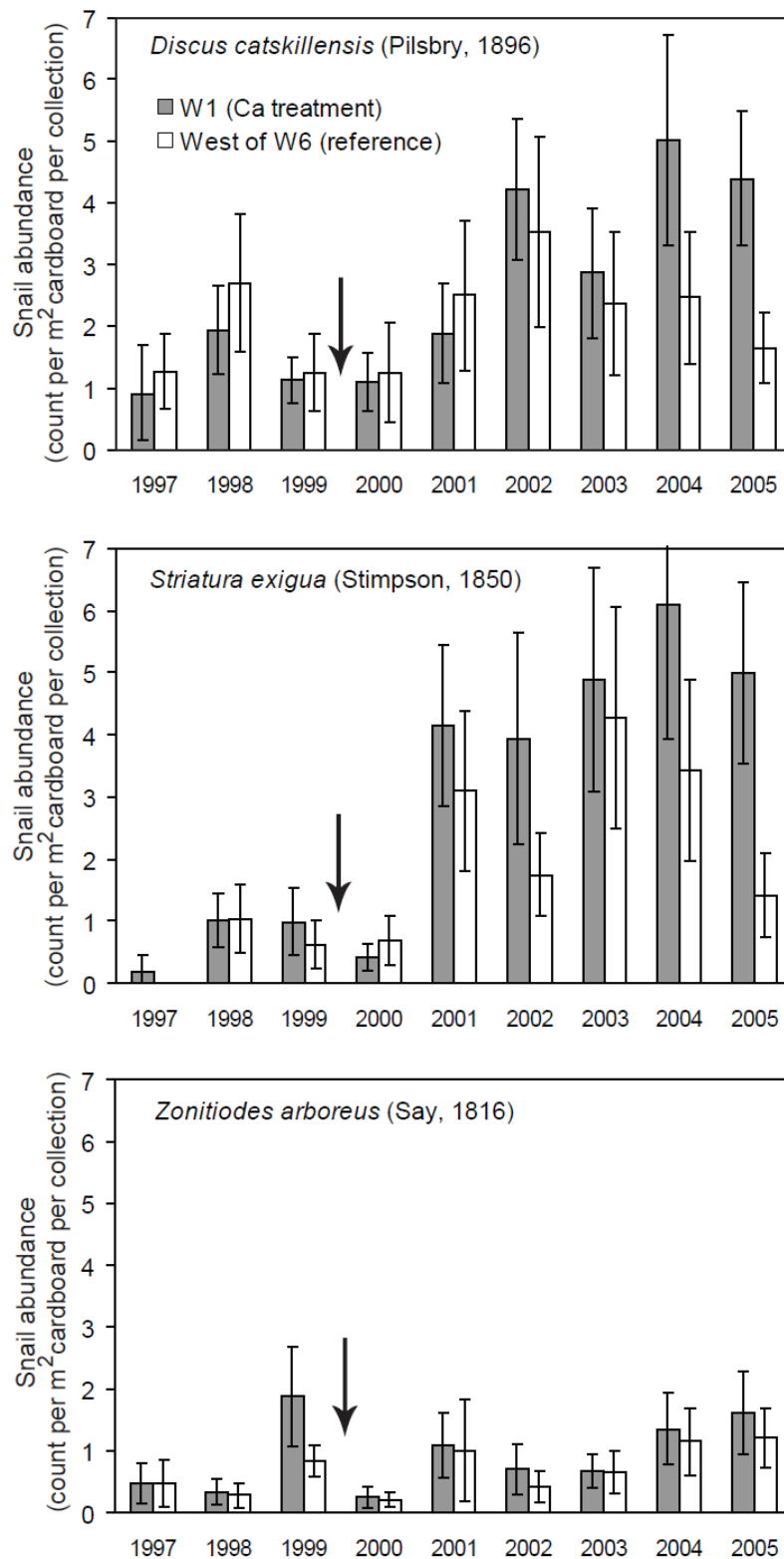


Figure 5. a) Ratios of Ca/Sr (molar) and $^{87}\text{Sr}/^{86}\text{Sr}$ in composite samples of understory vegetation (circles), overstory vegetation (triangles) and snail shells (asterisks, with dashed-line box encompassing all data) collected from W1 in 1999, prior to the Ca application. The solid-line box encompasses the range of predicted compositions of food ingested by the snails by assuming that the Ca/Sr discrimination factor (DF) ranges from 3 to 4 (see text) and that the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of food ingested includes the full range of values for vegetation. **b)** Ratios of Ca/Sr (molar) and $^{87}\text{Sr}/^{86}\text{Sr}$ in snail shells (diamonds) collected in 2005 as well as the region from A) encompassing the observed composition of snails and the predicted compositions of food ingested by the snails in 1999. Also plotted is the composition of the wollastonite applied to the watershed, and a series of mixing lines (see Dasch *et al.* (2007) for additional explanation of mixing relation on this type of diagram. Lines (a) and (b) bracket the region where simple mixtures of 1999 vegetation and wollastonite would plot, and where snails would be expected to plot if snails did not discriminate between Ca and Sr (i.e., DF=1). Lines (c) and (d) bracket the region where snails would be expected to plot, assuming a snail DF=3 and 4, respectively. The coincidence of the 2005 snail shell compositions with the region bracketed by lines (c) and (d) confirms that we can calculate the percentage of Ca in the snail shells that is derived from the wollastonite addition assuming that DF is between 3 and 4.

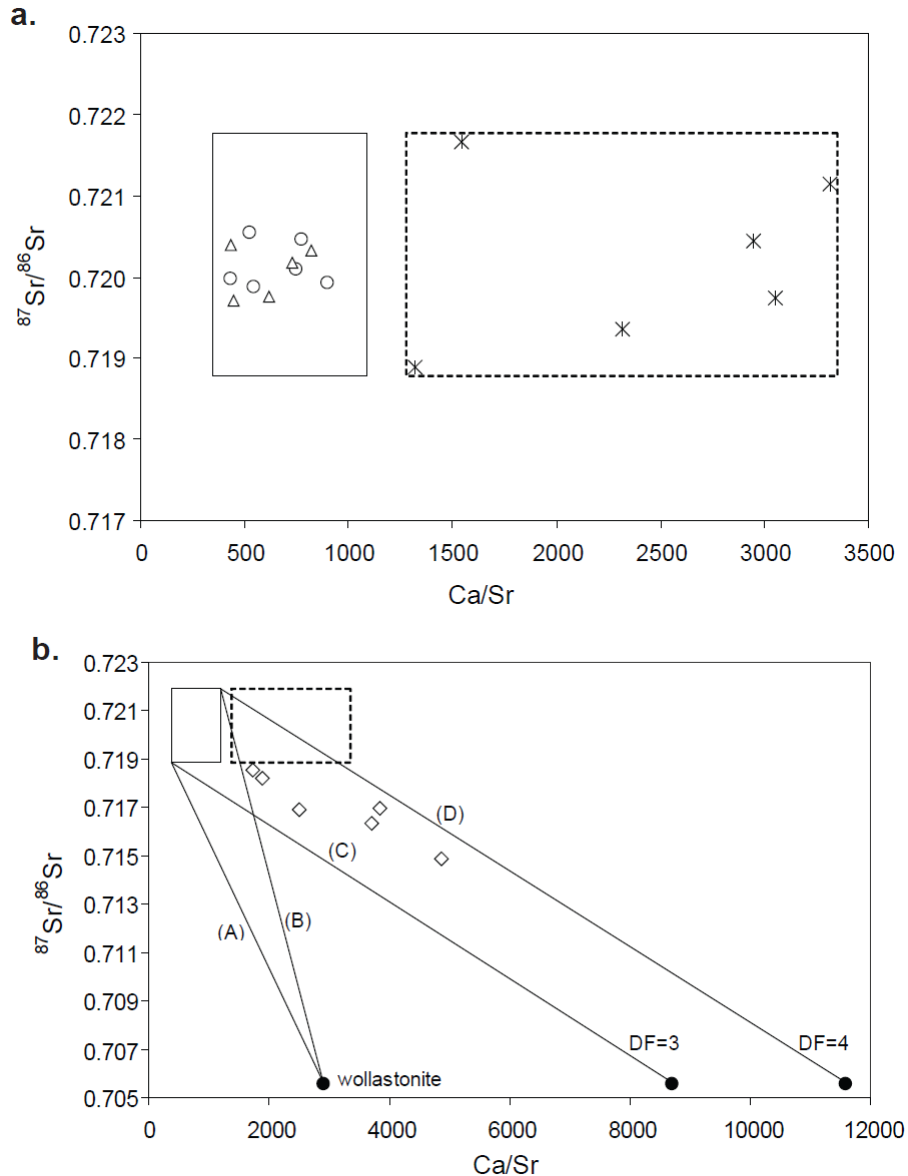


Figure 6. Snail abundance plotted against the relative importance value of beech within 5m of each of the 15 cardboards at each of the three elevations within the two watersheds, W1 and west of W6 of the Hubbard Brook Experimental Forest. The regression for cardboards on W1 (dashed line) is not significant but the regression for cardboards west of W6 (solid line) is significant.

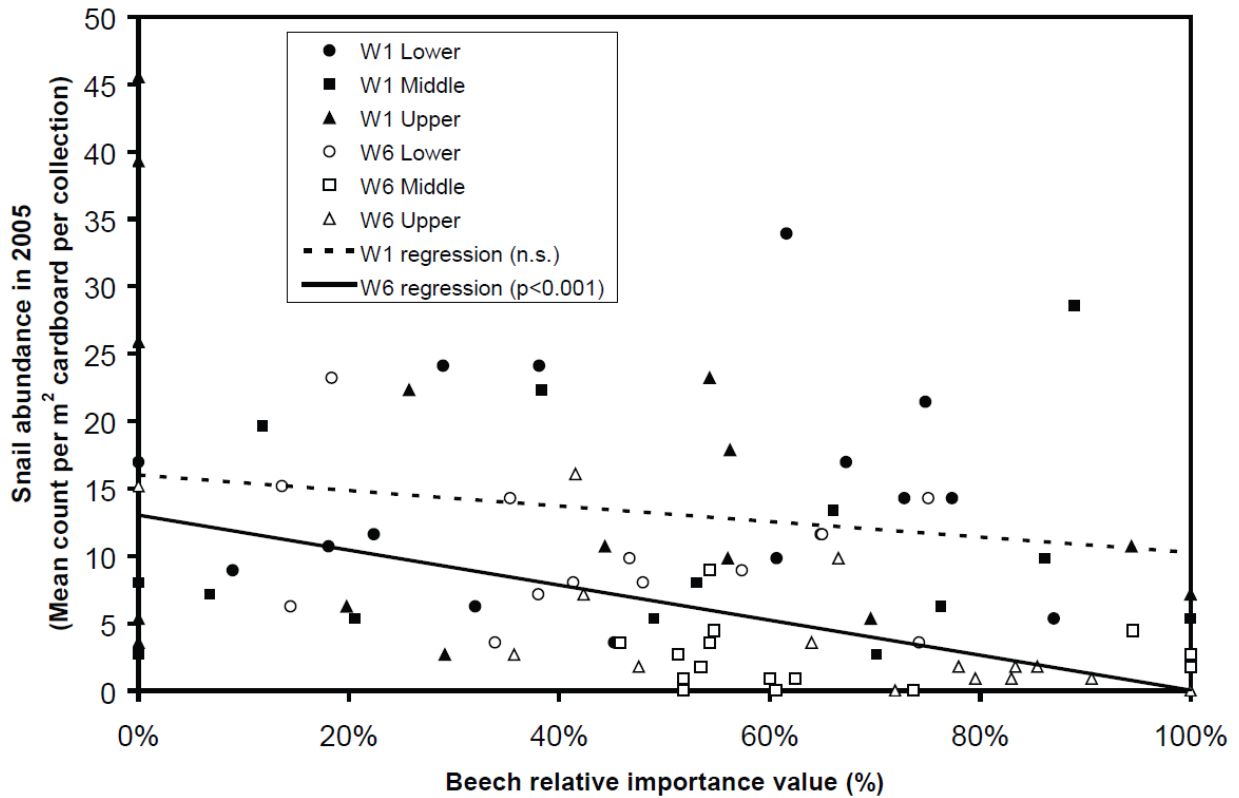


Figure 7. a) Mean snail abundance and **b)** species richness by watershed (W1 and west W6) and elevation, plotted against estimated Ca concentration in the Oi layer of the forest floor for the Hubbard Brook Experimental Forest. Error bars show 95% confidence intervals of the mean on each axis.

