CALCIUM ISOTOPE DYNAMICS IN THE HUBBARD BROOK SANDBOX EXPERIMENTS

By

AMY DAWN CAREY

A thesis submitted in partial fulfillment of
the requirements of the degree of

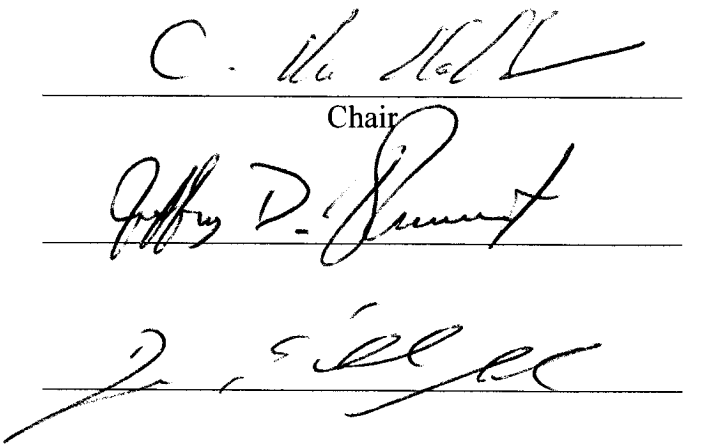
MASTER OF SCIENCE

WASHINGTON STATE UNIVERSITY
Department of Geology

AUGUST 2006

To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of AMY DAWN CAREY find it satisfactory and recommend that it be accepted.



Chair

ACKNOWLEDGEMENTS

The success of this research represents the culmination of efforts of many different people. Garret Hart and Jeff Vervoort went above and beyond the call of duty to help create the methods necessary for these isotope measurements to be made. I am forever grateful for the time, talent, and good humor they put forth to make this project a possibility. Charles Knaack ran many samples for me in the initial stages of this experiment and I appreciate the time and ingenuity it took to accomplish that, as well as all of the help he offered along the way. Tom Bullen, at the USGS office in Menlo Park, CA, also contributed his time and expertise to help get our methods working here at WSU. Zsuzsanna Balogh helped me run samples when this project was just starting, gave me tireless amounts of Hubbard Brook data whenever I discovered I needed it, contributed her ideas and expertise, and was just overall a great mentor and friend. C.Kent Keller, my advisor, has also been a great mentor and I will forever appreciate his patience as I tried to master the complexities of both the ecosystem and isotope portion of my research as well as the time and effort he committed to this project. I would like to thank all of my committee members, C.Kent Keller, Dirk Schulze-Makuch, and Jeff Vervoort for their efforts and ideas and especially for fitting me into their busy summer schedules. I would also like to thank the WSU Geology department who has generously supported me for the past two years. Most importantly I would like to thank my family and friends whose never-ending love and support are what got me through this with a smile on my face. Special thanks go out to my parents, who have always supported me in my academic pursuits and to Scott, who is amazing and whose support I could not have done this without.

CALCIUM ISOTOPE DYNAMICS IN THE HUBBARD

BROOK SANDBOX EXPERIMENTS

Abstract

by Amy Dawn Carey, M.S.
Washington State University
August 2006

Chair: C. Kent Keller

Storage and release of calcium from watersheds is controlled by biogeochemical processes in terrestrial ecosystems. The Hubbard Brook sandbox experiments allow researchers to monitor changes in all nutrient pools within ecosystems on watershed chemistry, including the biologic pool of nutrients stored in plant tissues. When calcium is absorbed for plant nutrition, calcium isotopes undergo stable mass fractionation. Discharge and soil water from two large 'sandbox' lysimeters were analyzed. One sandbox was planted with red pine trees that were harvested after 16 years of growth while the other became colonized by non-vascular vegetation. Isotopes were measured using MC-ICP-MS in the Washington State University GeoAnalytical Laboratory. Within the non-vascular sandbox there was no variation measured in $\delta^{42}Ca$ in both discharge and shallow soil water. The lack of variation in $\delta^{42}Ca$ in the non-vascular sandbox is attributed to chemical weathering releasing calcium from the system. Results also showed no variation in the $\delta^{42}Ca$ value of discharge and soil water within the red pine sandbox and between the red pine and non-vascular sandbox. This result is contrary

to previous findings that have measured up to a 1‰ variation in terrestrial ecosystems. When trees were alive in the red pine sandbox, discharge water was dominated by chemical weathering in the lower soil profile which would not have affected the $\delta^{42}Ca$ value. After harvest calcium was released into the system by nitrification of biomass and the buffering of H^+ by soil. The calcium from soil could have negated the contribution of 'light' calcium from biomass or the biomass was not fractionated. If the biomass is not fractionated, it would indicate that measurable fractionation of calcium in plants requires time and many bio-cycles to accumulate small initial fractionations. This concept of time-dependent fractionation is new but would explain the lack of variation observed in the sandbox ecosystems. It also indicates fractionation due to uptake by plants may be smaller than previously thought. Results support previous sandbox studies that have concluded that the hydrochemistry of water released from primary-successional ecosystems is controlled by CO_2 weathering and not affected by plant controlled nutrient cycling in the upper soil profile.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Dedication

To my Nana

Introduction

Calcium is a macronutrient, essential for the structure and function of life on earth as well as a major constituent of the rocks and minerals present in the earths crust. The global calcium cycle is intimately linked with both the global carbon and nitrogen cycles, two cycles that are being dramatically affected by human activities. Calcium is released from the continents through weathering and transported by surface waters where it ultimately arrives in the world's oceans. In the oceans calcium is sequestered into carbonates, effectively regulating atmospheric CO_2. The storage and release of calcium from continents is directly controlled by biogeochemical processes in terrestrial ecosystems. The rise of vascular plants in the Silurian and their effect on the weathering of the continents is thought to have significantly affected the concentration of CO_2 in the atmosphere (Moulton and Berner, 1998). Calcium cycling in terrestrial ecosystems, and its subsequent export from watersheds, can thus be linked to larger global nutrient cycles.

The same human activities that have altered the global carbon and nitrogen cycles have also led to the formation of acid rain. In the United States the effects of acid rain is a problem mostly limited to regions in the eastern part of the country. Deposition of acid rain into ecosystems in the northeastern U.S. have disrupted natural calcium cycles and subsequently leached large pools of available calcium from the soil profile where it is lost to ground or surface waters (Driscoll et al., 2001; Likens et al., 1998; Schaberg et al., 2001). Large losses of calcium are detrimental to ecosystem health because they can damage plant health (even to the point of death) and decrease the ability of soil to neutralize future acid inputs (Likens et al., 1998; McLaughlin and Wimmer, 1999). Calcium losses from acid rain input have increased the importance of understanding the natural behavior of calcium in ecosystems.

Calcium has many important roles in terrestrial ecosystem processes. The calcium ion is introduced to the ecosystem through the weathering of primary minerals and the influx of precipitation. Once released it can occupy cation exchange sites in the soil, form secondary minerals, or exist as a cation in the soil solution (Schaetzl and Anderson, 2005). Calcium in the soil solution can be absorbed by plant roots or leach from the soil profile through the downward movement of water (denudation) (Chapin III et al., 2002). Calcium that becomes part of the plant structure can return to the soil by leaching of the canopy and decomposition of the litter layer and fine roots, where it may once again be taken up into the plant. This 'bio-cycling' is a widely recognized process by which plants conserve nutrient supplies and control nutrient cycling within ecosystems.

The Hubbard Brook Ecosystem Study in New Hampshire has been investigating plants effects on watershed chemistry since 1963 by monitoring and manipulating small, natural watershed systems (Bormann and Likens, 1967; Likens, 2004; Likens et al., 1998). Hubbard Brook was one of the first areas to document the affects of acid rain on northeastern hardwood forests and has also made numerous contributions to ecosystem research by broadening it from its previously (limited) biological perspective. This new perspective views ecosystems as made up of a complex flux of nutrients between geological, soil, and biological pools. Plants are regarded mainly as weathering agents that can act as both a source or sink for nutrients in the system.

A detailed report and quantification on the sources, fluxes, and losses involved in the biogeochemical cycling of calcium within the watersheds at Hubbard Brook was published by Likens et al. (1998). Reports on the behavior of other major elements in the watersheds at Hubbard Brook have also been published. However, even with the use of small watershed

systems, it was still difficult to quantifiably assess and isolate the specific contributions from changes within the biologic, geologic, and soil pools to the chemistry, specifically of nitrogen, observed at the watershed outflow. To overcome these difficulties, small 'sandbox' lysimeter mesocosms were constructed in 1982 to provide researchers with the experimental control necessary to monitor all available nutrient pools (Bormann et al., 1987b). These experiments have generated questions about how the growth of vascular plants affects the weathering, sequestration, and hydrologic release of calcium and other macronutrient cations from simple ecosystems. Numerous investigations on nutrient cycling and flow patterns within the sandboxes have been published since their construction (Berner et al., 1998; Bormann et al., 1993; Bormann et al., 2002; Bormann et al., 1998; Keller et al., 2006; Keller et al., in press; O'Brien et al., 2004); but questions still remain concerning the influence of vascular plants on calcium behavior in the sandboxes.

Calcium isotopes are an innovative tool that is allowing researchers to explore calcium cycling in new ways. Calcium has six stable isotopes: ^{40}Ca, ^{42}Ca, ^{43}Ca, ^{44}Ca , ^{46}Ca, and ^{48}Ca (DePaolo, 2004). The relatively large mass differences in this isotope system cause stable mass fractionation when the ion is absorbed for plant or animal nutrition because the lighter isotopes are preferentially taken up into the organism (DePaolo, 2004; Platzner and Degani, 1990; Skulan and DePaolo, 1999; Skulan et al., 1997). Studies in terrestrial and marine ecosystems have found that organisms become progressively enriched in light calcium with each succession on the food chain (DePaolo, 2004; Schmitt et al., 2003; Skulan et al., 1997; Zhu and MacDougall, 1998). All organisms are therefore recognized to be isotopically lighter than their calcium source.

Studies of calcium isotope dynamics in terrestrial ecosystems are limited but findings have shown that the plants are indeed enriched in light calcium, which in turn has been determined to affect the isotopic composition of calcium within different soil pools in the rooting zone (Bullen et al., 2004; Perakis et al., 2006; Schmitt et al., 2003). Up to a 1‰ variation in $\delta^{42}Ca$ has been observed in ecosystems due to biologic processing of calcium (Bullen et al., 2004; Perakis et al., 2006; Schmitt et al., 2003). Calcium isotope behavior in ecosystems like the Hubbard Brook sandboxes should consequently also be controlled by biologic processes. This paper will focus on the calcium isotope dynamics within the sandbox mesocosms at Hubbard Brook as a means to explore the biotic processes affecting the behavior of calcium in ecosystems.

The sandboxes at Hubbard Brook provided the perfect experimental set-up to explore such behavior because they were designed so that the only variable between different sandboxes was vegetation cover. The research plan was to analyze the effects of plants on calcium isotopes by comparing calcium isotope measurements from soil and discharge water in a sandbox with a limited covering of non-vascular vegetation to a sandbox planted with pine trees. It was expected that in the sandbox containing the pine trees the calcium would become enriched in heavy isotopes due to the uptake of light calcium into the tissue of the growing trees. After the trees were harvested and removed from the sandbox, it was hypothesized that the lighter calcium previously stored in the trees would appear in the soil and drainage waters through decomposition of the litter layer and below ground biomass. Calcium isotope ratios in the non-vascular sandbox were predicted to show no change with time due to the minor contribution of vegetation to processes within the ecosystem.

Although ecosystem studies have attributed changes in the calcium isotopic composition of both soil and discharge waters to the effects of plants, a direct comparison of calcium isotope behavior between two ecosystems that differ only in vegetation has never been made and is uniquely possible with the sandbox experiments. Changes in calcium isotopes due to tree growth and harvest have also never been monitored or reported previously. Measuring the calcium isotopes in discharge water from this experiment should provide a means to track the effects of the biological pool on calcium exported from this simple ecosystem. This, in turn, provides a unique perspective on the influence of the biological calcium pool in terrestrial ecosystems over the calcium released from ecosystems to oceans and, in a larger part, contributes insight on the control of plants over the global calcium cycle.

Methods

Study Location

The sandboxes are located in the Hubbard Brook Experimental Forest in the White Mountains of north-central New Hampshire, USA. They were constructed in 1982 in a topographically low area within the watershed (O'Brien et al., 2004). This study focuses on only two of the larger sandboxes (7.5 x 7.5 x 1.5 m deep) (Bormann et al., 1987a). Excavations of original soil material were lined with impermeable Hypalon geomembrane, layered with 15 cm of gravel, and filled to the top with granitic glaciofluvial sand. Native topsoil (5 cm) was rototilled into the top 20 cm of sand (Bormann et al., 1987a). Slotted polyvinylchloride pipe was placed in the gravel layer before the box was filled to collect drainage water from the sandbox.

One sandbox was actively weeded of vascular plants until it was colonized by lichen (*Cladonia cristatella* Tuck.) and a moss (*Polytricum* spp.) (Bormann et al., 1987a). The other

sandbox was planted with 196 red pine seedlings (*Pinus resinosa* Ait.) in a grid that provided each tree with 0.25 m^2 of growing space (Bormann et al., 1987a). These sandboxes are referred to as the non-vascular and red pine box, respectively. The boxes were then left relatively undisturbed except for the installation of sampling equipment, soil sampling, the occasional weeding, and the replanting of a few failed red pine seedlings (Ingersoll et al., 1987). After 16 years of development, the red pine trees were harvested on May 1, 1998. The above ground biomass (trunk, branches, etc) was removed by hand and using wooden planks to minimize disturbance to the soil. The biomass that was left included stumps, roots, and a 5-8 cm thick litter layer (Keller et al., in press).

Data Collection

Although many different types of samples have been collected for the sandbox experiments, this study only analyzed samples of soil and discharge water. Drainage water was collected from the pipe installed into the gravel layer in the sandbox, as described previously. Soil water samplers were installed in 1995 at 15, 35, and 95 cm depth with three samplers installed at each depth. Each sampler consisted of a fritted glass plate on a circular collection chamber connected to a vacuum pump (O'Brien et al., 2004).

Water samples were filtered through a 0.2 μm nylon filter in the field. One aliquot was frozen for anion analysis and another acidified to below a pH of 2 using HCl for cation analysis. Discharge water was measured for major anions and cations at Yale University using ICP (inductively coupled plasma spectroscopy) and IC (ion chromatography), respectively . Soil water samples were measured for anions on IC and cations were measured by IC (1996-1997) and ICP (1998-2000) (Havig, 2002; Keller et al., 2006; O'Brien, 2000). A collection of water

samples from the sandbox experiments have been stored refrigerated since collection in nalgene bottles at the University of Vermont, which is where this study obtained samples for analyses.

There were four major objectives when choosing samples from the sandbox collection for analyses in this study. Samples from both the non-vascular and red pine box were chosen so that the non-vascular box would act as a control by providing the chemical signature of waters not influenced by tree growth. Samples from the beginning of the experiment (1985) to the most recent samples (2003) were chosen to provide a look at the evolution of the chemistry over the length of the experiment. More samples were chosen from the red pine box, and specifically samples from after the trees were harvested, to obtain a detailed look at the behavior of calcium after vascular plant growth had been eliminated and decomposition was releasing high concentrations of Ca^{2+} into soil and discharge waters. This variety of samples will allow a complete comparison of the effects of the trees on calcium isotope measurements over time in the sandboxes. Lastly, many of the samples were chosen because strontium isotope analyses had been previously performed on these samples, which would allow for future comparisons between these two isotope systems.

Column Chromatography

The first step in the procedure developed to measure calcium isotopes was the separation of calcium from the other elements present in the water sample using column chromatography. Between 5-12 milliliters of water were used depending on concentration of calcium in the sample. Samples were evaporated to dryness, redissolved in twice distilled and titrated 2M HCl, sonicated, centrifuged, and loaded into ~1cm diameter quartz columns filled with 8.9 mL Bio-RAD AG 50W-X8 (200-400 mesh) cation exchange resin (modified from (Tera et al., 1970). Twice distilled and titrated 2M HCl was used to collect the calcium fraction (see App. A for full

procedure). The timing of calcium release from the columns was determined on an ICAP Spectrophotometer and the results were checked on an HP 4500 ICP-MS (App. A). A 100% yield of the calcium fraction was obtained so that there was no fractionation of the sample on the column (Russell and Papanastassiou, 1978). The calcium fraction was then processed through a different set of columns to remove any strontium still present in the sample. This second step uses 3mm x 33mm Teflon columns filled with Eichrom Tech. Inc Sr Spec resin (see App. A for full procedure). Analyses on the HP 4500 ICP-MS showed that these columns effectively removed ~95% of the minor amount of strontium left in the calcium fraction. The removal of the remaining strontium is necessary to mitigate interferences from strontium on the mass spectrometer.

A procedural blank was processed during each set of sample separations. The blanks contained an average of ~0.08 ppm calcium, which is ~1.6% of the concentration of calcium in the sample and thus considered to be negligible. Samples were prepared directly before analysis on the mass spectrometer to be run at 5 ppm of calcium dissolved in 2% of HNO_3. Each sample was also spiked with 50 ppb of titanium from an ICP-MS titanium standard (High Purity Standards: Cat. #100062-3, Lot #202125).

Analysis

Calcium isotopes were measured on a Finnigan Neptune MC-ICP-MS (multicollector-inductively coupled plasma-mass spectrometer) housed in the Washington State University GeoAnalytical Laboratory. Currently, only limited publications exist on procedures for this type of analysis due to complications from interferences, as discussed below (Halicz et al., 1999; Wieser et al., 2004). Therefore, a the major part of this research was developing the methods

necessary to accurately measure the calcium isotopic abundances present in the target samples because these procedures had not been previously set-up at WSU.

The natural abundances of the stable calcium isotopes are as follows: ^{40}Ca (96.98%), ^{42}Ca (0.642%), ^{43}Ca (0.133%), ^{44}Ca (2.056%), ^{46}Ca (0.003%), and ^{48}Ca (0.182%). Titanium has the following five stable isotopes with corresponding abundances: ^{46}Ti (8.249%), ^{47}Ti (7.437%), ^{48}Ti (73.720), ^{49}Ti (5.409%), ^{50}Ti (5.185%) (Rosman and Taylor, 1998). Samples were spiked with titanium to correct for mass bias, which will be discussed in the following section. The overlapping mass ranges of calcium and titanium allow them to be measured consecutively in the mass spectrometer during analyses. There are several interferences in the calcium mass range that complicate the analysis of calcium isotopes and had to be taken into account when developing the procedure on the MC-ICP-MS.

The MC-ICP-MS uses argon plasma to ionize the samples. There is an isobaric interference on ^{40}Ca (39.9626 amu) from ^{40}Ar (39.940 amu). This extremely small mass difference is indistinguishable even at high resolution, so ^{40}Ca was not included in our analysis. ^{40}Ca has recently been measured on an argon plasma MC-ICP-MS by running the machine in cool plasma mode, which reduces the plasma energy to a point that the isobaric interferences are insignificant, but this was not attempted for this study (Fietzke et al., 2004). The target ratio for this study was consequently ^{44}Ca/^{42}Ca. It is usually preferred to measure the most abundant isotope, which is ^{40}Ca at 96.98% abundance for calcium. However, ^{40}Ca is also a product of decay of ^{40}K so it is possible for a sample to be enriched in ^{40}Ca from radiogenic decay. The ^{44}Ca/^{42}Ca ratio measured for this study would only vary due to stable isotope fractionation.

Carbon, oxygen, nitrogen, and hydrogen can all form possible molecular interferences on the target isotopes ^{44}Ca and ^{42}Ca. These possible interferences include $^{40}Ar^{1}H_{2}^{+}$ and $^{40}Ar^{2}H^{+}$ on

^{42}Ca and $^{14}N_2{}^{16}O^+$, $^{12}C^{16}O_2{}^+$ on ^{44}Ca. Our approach was to use a desolvating nebulizer for sample introduction in order to reduce the amount of C, O, and H introduced with the sample to make these interferences insignificant.

Interferences with doubly-charged ions in the calcium mass range could occur from $^{84}Sr^{2+}$, $^{86}Sr^{2+}$, and $^{88}Sr^{2+}$ on ^{42}Ca, ^{43}Ca, and ^{44}Ca, respectively. Inaccuracies resulting from this interference were mostly mitigated by removing the majority of strontium with column chromatography. Strontium interferences were monitored for during the analysis by measuring the intensity on mass 43.5 ($^{87}Sr^{2+}$ has a mass to charge ratio of 43.5) (Halicz et al., 1999). However, analyses produced no measurable intensity on 43.5, so interference from strontium is negligible.

The final procedure was to run the Neptune at low resolution using a CETAC Aridus desolvating nebulizer system to introduce the sample. The results for each sample are the average of forty measurements taken within one analysis on the Neptune. During each measurement, the magnet first directs selected calcium isotopes into the faraday cups and then does a second sweep to measure titanium isotopes (Figure 1). The mass difference between the target calcium isotopes (^{42}Ca, ^{43}Ca, ^{44}Ca) and titanium isotopes (^{47}Ti, ^{49}Ti) is too high to measure them simultaneously on this machine.

Both internal and external calcium isotope standards were analyzed. The internal standard was created by dissolving Alfa Aesar Calcium crystalline dendritic pieces in dilute nitric acid (Stock #14457, Lot #J12Q044, 99.98% metals basis). The external standard was seawater collected from the northern Pacific Ocean. The calcium isotopic composition of modern seawater has been determined to be uniform, with a $^{44}Ca/^{42}Ca = 3.2719$ (Hippler et al., 2003; Schmitt et al., 2001). The internal standard was calibrated using seawater and determined

to be $^{44}Ca/^{42}Ca = 3.2700$. The internal standard was run between every sample and seawater was run as an unknown sample at the beginning and end of each session.

Modern seawater was analyzed 27 times over the 5 month span of the study to determine the external reproducibility of the analyses (Figure 2). The results are $^{44}Ca/^{42}Ca = 3.2719 \pm 0.0007$, where the uncertainty is two standard deviations of the 27 analyses. This variability produces an uncertainty of ±0.20‰ in each sample analysis (2σ at 95% confidence level) (Eqn.1).

$$error = \left[\frac{A}{B}\right] * 1000 \qquad (1)$$

Where: A = analytical uncertainty (0.0007)

B = average of all corrected seawater measurements

Seawater was sometimes the only sample that was analyzed on a given day. If uncertainty is calculated from the average of seawater measurements taken on days that also included Hubbard Brook samples (14 seawater measurements), the uncertainty is slightly smaller at ±0.16‰ (2σ at 95% confidence level).

Data Reduction

Standards and samples were spiked with titanium prior to analysis to determine the mass bias correction. The titanium spike provides an internal isotopic standard that can be used to correct for the instrumental bias toward heavier isotopes. However, the mass bias correction factor for titanium is not identical to the correction factor for calcium. During analyses the mass bias between titanium and calcium exhibited a constant linear relationship on a plot of $\log(^{49}Ti/^{47}Ti)$ v. $\log(^{44}Ca/^{42}Ca)$, which allows the correction factor for calcium to be calculated from the correction factor for titanium (App. B). The procedure for this correction was modified from a similar procedure published for correcting lead isotope ratios using thallium isotopes

(White et al., 2000). After correcting for mass bias, the data were normalized to the value for the internal standard (App. B).

Mass bias corrections using titanium were determined to be successful due to the reproducibility of the seawater standard. However, problems with this method arose on days that the mass bias relationship between titanium and calcium did not appear linear. It was found that simple sample-standard-sample bracketing corrections could be applied with the same results as the titanium procedure. This type of correction applies the average of the correction factors for the standard run on either side of the sample to the sample ratio to determine its correct value. Therefore, both procedures were applied to reduce the data depending on the behavior of the machine the day the samples were analyzed.

The mass bias corrected $^{44}Ca/^{42}Ca$ ratio in the sample was then used to calculate the reported value, $\delta^{42}Ca$ (App. B). The notation $\delta^{42}Ca$ is used instead of $\delta^{44}Ca$ because in the majority of the literature, $\delta^{44}Ca$ refers to a value calculated using the $^{44}Ca/^{40}Ca$ ratio (Schmitt et al., 2003). The $\delta^{42}Ca$ of the samples is reported in a per mil notation from seawater in compliance with much of the literature, although several references use the standard NIST SRM 915a to calculate their delta values. The delta values of samples can be easily converted to the different reference standards using the equations reported in the literature (Eisenhauer et al., 2004; Hippler et al., 2003). $\delta^{42}Ca$ can also be converted to the more commonly used $\delta^{44}Ca$ value by using the equation provided in the literature (Eqn.2) (Eisenhauer et al., 2004).

$$\delta^{44}Ca = \delta^{42}Ca * 1.9995 \qquad (2)$$

Results

A total of 28 samples from the Hubbard Brook sandbox experiments were analyzed for calcium isotopes. Nineteen samples came from the red pine sandbox, including 15 drainage water samples, 3 shallow soil water samples, and one deep soil water sample. Nine samples were measured from the non-vascular sandbox, 8 from discharge water and one from shallow soil water. Results show no variation in the $\delta^{42}Ca$ value in discharge water outside of the 0.16‰ error. The $\delta^{42}Ca$ values did not vary either between the red pine and non-vascular box or over time within either box (Figure 3, 4&5). This lack of variation is independent of changes in the calcium concentration within the discharge water. Soil water samples also showed no variation in the $\delta^{42}Ca$ value both with depth in the red pine sandbox or between the red pine and non-vascular shallow soil water samples (Figure 3&4). The non-vascular shallow soil water sample has the lightest $\delta^{42}Ca$ value measured in all the 28 samples and non-vascular discharge water from 9/14/1999 has the heaviest $\delta^{42}Ca$ value. The average value of $\delta^{42}Ca$ for red pine discharge water was -0.40‰ and for non-vascular discharge water was -0.34‰. There are a limited number of samples that have different $\delta^{42}Ca$ values outside of the analytical error. Discharge waters from 9/14/99 in both the red pine and non-vascular sandbox and 10/8/87 in the red pine box have heavier $\delta^{42}Ca$ values than red pine discharge water on 7/13/99 and non-vascular shallow soil water on 5/21/99 (Figure 4).

Discussion

Non-vascular sandbox

The drainage water in the non-vascular sandbox is expected to carry the calcium isotopic signature of sandbox materials weathered without biotic influence because weathering processes within the non-vascular sandbox should be dominated by inorganic chemical weathering due to experimentally restricted vegetation growth. Calculations show relatively equal amounts of weathering and denudation in the non-vascular sandbox over the course of the study, which indicates that most weathering products leave the sandbox without being stored in significant amounts in soil or other ecosystem pools (Figure 6) (Balogh, 2006). The calcium isotopic composition of the drainage waters in the non-vascular sandbox shows no significant variation over the length of the study period (Figure 4). This was expected because previous studies have only attributed significant variations of $\delta^{42}Ca$ in terrestrial ecosystems to the biological processing of calcium (Bullen et al., 2004; DePaolo, 2004; Perakis et al., 2006; Schmitt et al., 2003; Skulan and DePaolo, 1999; Skulan et al., 1997; Zhu and MacDougall, 1998). Soil water measured at 15cm depth has a calcium isotopic composition that is not significantly different from that of the drainage waters (Figure 4). Drainage and soil water measurements within the non-vascular box thus indicate no influence of biota on the calcium isotopic composition and agree with the previous findings that the waters in the non-vascular box are solely products of chemical weathering of sandbox materials (Keller et al., 2006).

Red Pine sandbox

Red pine sandbox development can be divided into three different stages based on the influence of the trees on processes within the sandbox (Balogh, 2006). During the first five years

of monitoring (1983-1988) the trees were growing rapidly. Weathering in the sandbox was much greater than denudation (Figure 6), as most weathering products were being retained in biomass and soil pools (Balogh, 2006). It was hypothesized that during this time the discharge water would have a heavier calcium isotopic signature than the non-vascular box because the biomass would have preferentially retained light calcium isotopes. Results show that drainage water in the red pine sandbox is not isotopically different from the non-vascular box and does not vary with time/growth of the trees (Figure 3&5). These results suggest that the discharge water at this time is dominated by chemical weathering in the lower part of the soil profile, a conclusion also deduced independently by Keller et al. based on changes in the calcium concentration within the soil profile (2006). Any influence of tree growth on the calcium isotopes in discharge water is either too small to measure and/or the isotopically fractionated calcium has been retained in the soil profile.

The second stage of development within the red pine box was marked by a ten year period (1988-1998) of reduced biomass accumulation (tree growth). The weathering and denudation fluxes were also both reduced in magnitude from the previous stage to values that were relatively equal (Figure 6). This indicates the trees were obtaining their nutrient requirements mainly through bio-cycling in the upper soil profile (Balogh, 2006). Calcium isotope values for the discharge water during this time period are not different from the previous stage of development or from the non-vascular box (Figure 3&5). This supports the idea that during this time the discharge water was chemically dominated by chemical weathering in the lower soil profile, as it was during the first stage of tree growth. Any effect of nutrient cycling in the upper soil profile had no measurable effect on the calcium isotope measurements in the discharge water.

The third stage of development in the red pine sandbox occurred after the harvest of the trees, May 1, 1998. This disturbance increased the denudation although there was little/no increase in weathering, indicating that there had to be some other source of Ca^{2+} generation (Figure 6) (Balogh, 2006). Possible sources include both decomposing biomass (roots and litter left in the sandbox after the harvest) and/or soil pools. Elevated concentrations of calcium did not appear in the discharge water until a year after the harvest of the trees (Figure 3). The counter ion for the Ca^{2+} in this pulse was nitrate (NO_3^-) (Havig, 2002). This release of calcium and nitrate has also been observed in stream water after large scale harvesting in experimental Hubbard Brook watersheds (Likens et al., 1998). The appearance of large amounts of nitrate indicated a shift from a nitrogen limited to a carbon limited system within the sandbox (Keller et al., 2006). In this carbon limited system, nitrifying microbial communities decomposed biomass, which produced the large concentrations of nitrate in the discharge water. The appearance of calcium and nitrate together in the discharge water suggested that the majority of the calcium must be coming from decomposing biomass, with the litter layer contributing more than the roots (Havig, 2002). Calcium is a major component of more recalcitrant woody tissue and cell walls, so it is reasonable to conclude that this type of biomass would take longer to decompose and contribute to the solutes in the discharge water (Keller et al., 2006). However, upon closer inspection the process cannot be so straightforward. Nitrification produces H^+, but during the nitrate pulse there was no change in pH measured in the discharge water. The extra hydrogen ions produced during nitrification had to be buffered by the soil system. Soils buffer acid inputs by exchanging cations (such as calcium) for H^+ in different soil pools (Schaetzl and Anderson, 2005). The lack of pH change during nitrification then establishes that the calcium released from

the sandbox and into discharge water during the pulse came not only from decomposing biomass but also from soil pools.

We expected that the contribution of biomass to the calcium in discharge water after the tree harvest would make the calcium isotopic composition lighter than previous measurements taken when the discharge water was dominated by the products of chemical weathering. In fact, this project initially intended to use the amount of fractionation in the discharge water as an indicator of the contribution of biomass to the denudation of calcium after the harvest. However, results show no change in $\delta^{42}Ca$ during this time period as well as no change from the previous measurements in the red pine or non-vascular box (Figure 3&5). Soil water from the upper (15cm) part of the profile showed peak concentrations of calcium during this time but no significant difference in $\delta^{42}Ca$ from the discharge water nor from soil water in the deep part of the profile (95cm) (Figure 3) (Havig, 2002).

There are two possible interpretations that can be drawn from the lack of change of $\delta^{42}Ca$ in drainage and soil waters during the disturbance within the red pine sandbox. Studies that have measured the calcium isotope composition of plant biomass in ecosystems have established it to be lighter than the calcium in soil parent materials by 0.5 - 1‰ for $\delta^{42}Ca$ values (Perakis et al., 2006; Schmitt et al., 2003). We could assume that is true in the sandbox. However, nitrification released both biomass and soil calcium into the system at the same time so that the discharge and soil waters were a mixture of calcium from these two sources. The contribution of calcium from the soil pools could be large enough to dilute the lighter isotopic signature of the biomass calcium to a value undistinguishable from those before the Ca^{2+}-NO_3^- pulse. In fact, there is at least 4 times the amount of calcium available for release in the exchangeable soil pool compared to the biomass (Havig, 2002). Calculations that estimate how much calcium could be attributed

to decaying biomass indicate that the contribution from biomass could be as much as 80% of the total calcium released, which means about 20% of Ca^{2+} is from soil pools (App. E). These calculations first estimate the amount of biomass that decomposed from the amount of CO_2 released from the sandbox (Keller et al., in press) and then calculate the amount of calcium coming from biomass using previous estimates of the calcium concentration in plant tissue. These calculations could support the idea that there was no change in $\delta^{42}Ca$ because calcium from the soil pool effectively negated the contribution of light calcium from biomass.

The lack of variation in $\delta^{42}Ca$ during this time, even though there was a contribution of calcium from biomass, could also indicate that the biomass was not measurably enriched in light calcium isotopes compared to its sandbox source of calcium. This would be different from what has been reported in the literature, but key differences in the sandbox experiment make this a possibility. The most notable difference between the sandbox experiments as compared to the terrestrial ecosystems in which the previous calcium isotope studies took place is the extent of ecosystem and soil development. The red pine sandbox had very young trees grown in unweathered sediment materials in which no real soil profile had developed. The ecosystems in the literature on which calcium isotopes have been studied have presumably developed through the Holocene (Bullen et al., 2004; Perakis et al., 2006; Schmitt et al., 2003). This could indicate that a measurable degree of calcium isotope fractionation within an ecosystem requires time to develop. Time would allow very small (undetectable) fractionation due to biologic processing of calcium to be repeated through bio-cycling and propagate to measurable differences from the source material, both in the biomass and shallow soil pools. If this is in fact the case, no variation in the $\delta^{42}Ca$ existed in the sandbox experiments because there had not been enough time for bio-cycling of calcium to produce fractionation that was detectable.

More work can be done to refine the interpretations of calcium isotope dynamics in the sandbox experiments. Samples of both biomass and sandbox matrix materials were collected during the experiment and are available for further research. Measuring the calcium isotopic composition of these materials, particularly the biomass, would clarify the reasons for the lack of variation in $\delta^{42}Ca$ observed throughout the experiment.

Statistical Variations

Close inspection of the results reveal that there are a few data points that are statistically different from one another in both the non-vascular and red pine sandbox. Discharge water from 9/14/99 in both the red pine and non-vascular sandbox and 10/8/87 in the red pine box have heavier $\delta^{42}Ca$ values than red pine discharge water on 7/13/99 and non-vascular shallow soil water on 5/21/99 (Figure 3&5). The timing of these variations might indicate seasonal influences, possibly due to changes in flow rates because of an increase or decrease in precipitation. Large infiltration events have been determined to hydraulically activate small pores that have had more time to react with the surrounding material (O'Brien et al., 2004). These small pores could possibly be contributing water with a slightly heavier calcium isotopic signature. However, the data do not clearly suggest such a relationship (Figure 7).

One other notable variation is the apparent lightness of the shallow soil water in the non-vascular sandbox compared to the discharge water (Figure 3). Although the differences still appear to be within error (if only slightly), they could hint at an effect from the original soil material that was rototilled into the upper 20cm of each sandbox. Further characterization of the $\delta^{42}Ca$ in different sandbox materials is needed to definitively discuss the source and possibility of any of these variations.

Summary and Conclusions

The purpose of this research was to document dissolved calcium isotope variations in waters moving through Hubbard Brook sandboxes, and to use these variations to determine the extent of biologic influence on the behavior of calcium in these ecosystems. Calcium isotope composition were measured in discharge and soil water samples in both the non-vascular and red pine sandboxes spanning the length of the Hubbard Brook sandbox experiment. The non-vascular sandbox showed no variation in $\delta^{42}Ca$ which is consistent with a system dominated by inorganic chemical weathering processes. The red pine sandbox is a system where biological processes are believed to control the weathering and denudation in the ecosystem and these processes were predicted to result in variations in calcium isotopes. However, $\delta^{42}Ca$ showed no significant variation in the red pine sandbox waters throughout the experiment and was the same as values from the non-vascular box. When the trees were alive in the red pine sandbox, we suggest that the discharge water was dominated by chemical weathering processes in the lower soil profile and the calcium isotope values were unaffected by tree uptake and cycling of calcium. One year after the trees were harvested, the system was flooded with calcium and nitrate due to nitrifying decomposition and the release of calcium from soil pools. Previous studies have determined that biomass has a lighter $\delta^{42}Ca$, so the contribution of biomass to the calcium in the system was expected to 'lighten' the $\delta^{42}Ca$ value in the soil and discharge waters. However, $\delta^{42}Ca$ values did not vary significantly during this time and were the same as previous measurements within the red pine and non-vascular sandboxes.

There are two possible explanations for these results. The contribution of dissolved calcium from the soil pools could have diluted the lighter isotopic signature of the calcium from biomass to a point that no differences in the $\delta^{42}Ca$ could be measured. Also, it could be that

measurable biologic fractionation requires many cycles of plant uptake and release, repeated on the timescale of soil development. This concept of time being a factor in measurable amounts of calcium isotope fractionation in ecosystems has not been previously presented in the literature. If this concept is true, it may be that the amount of calcium isotope fraction that occurs in ecosystems over time is known, but fractionation of calcium due to uptake by plants is smaller than previously thought and has not yet been sufficiently quantified. Regardless, these results underline interpretations of recent Hubbard Brook sandbox studies that have concluded that the hydrochemistry of water leaving primary ecosystems is controlled by CO2 weathering (Keller et al., 2006).

The control the biological pool of calcium has over the sequestration and release of calcium from simple ecosystems relates on a larger scale to the involvement of plant growth in the global calcium cycle. Previous research has established biologically mediated fractionation of calcium as the major cause for natural variations in calcium isotope ratios in terrestrial ecosystems. This research produced results contrary to previous studies because no variation was measured in calcium isotope ratios in the simplified sandbox ecosystems, indicating that measurable fractionation of calcium in ecosystems could be more complex than previously thought. Further characterization of biomass and other calcium pools within the sandbox materials is necessary to develop a more complete understanding of calcium isotope dynamics in the Hubbard Brook sandbox experiments.

Table 1: Corrected seawater standard values (App.D).

Date	$^{44}Ca/^{42}Ca$
1/24/2006	3.2721
	3.2724
	3.2718
	3.2718
	3.2718
	3.2720
	3.2719
	3.2718
1/31/2006	3.2723
	3.2726
	3.2725
2/24/2006	3.2718
	3.2719
3/8/2006	3.2713
	3.2724
	3.2722
	3.2710
	3.2720
3/10/2006	3.2720
	3.2720
4/18/2006	3.2718
5/2/2006	3.2718
	3.2719
5/3/2006	3.2718
5/4/2006	3.2719
	3.2719
	3.2718

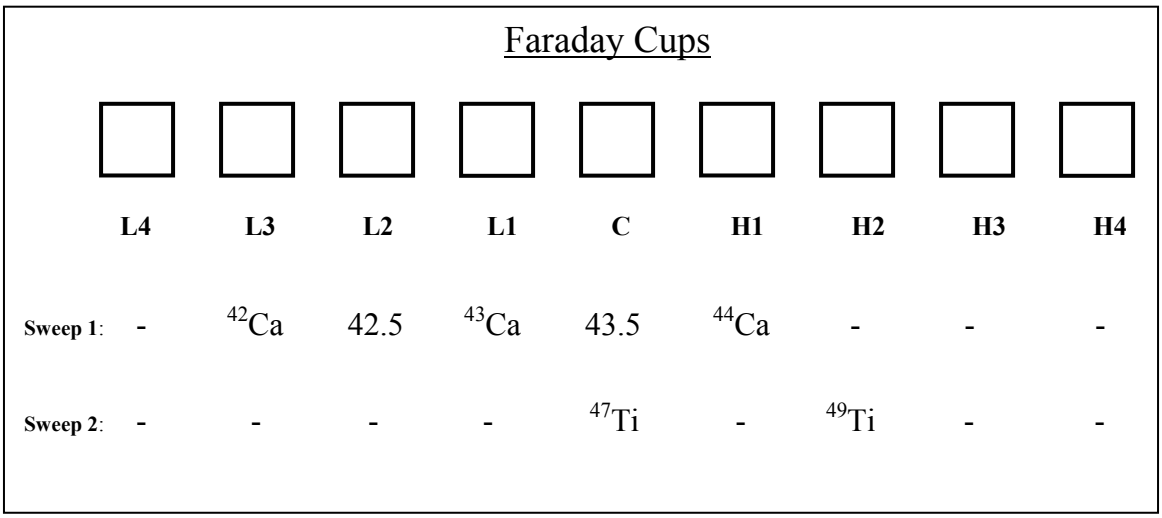


Figure 1: Collection configuration for measuring calcium isotopes with titanium spike on the WSU ThermoFinnigan Neptune MC-ICP-MS.

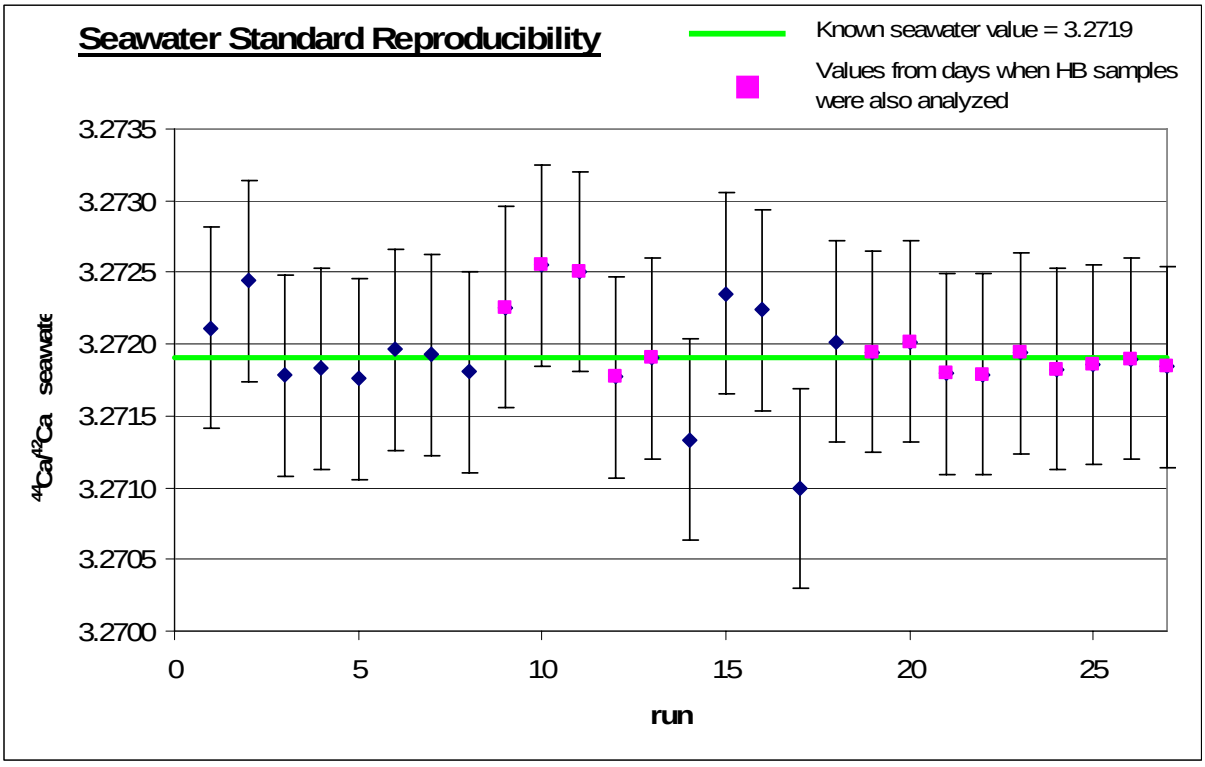


Figure 2: Seawater standard reproducibility shown in chronologic order (Table 1).

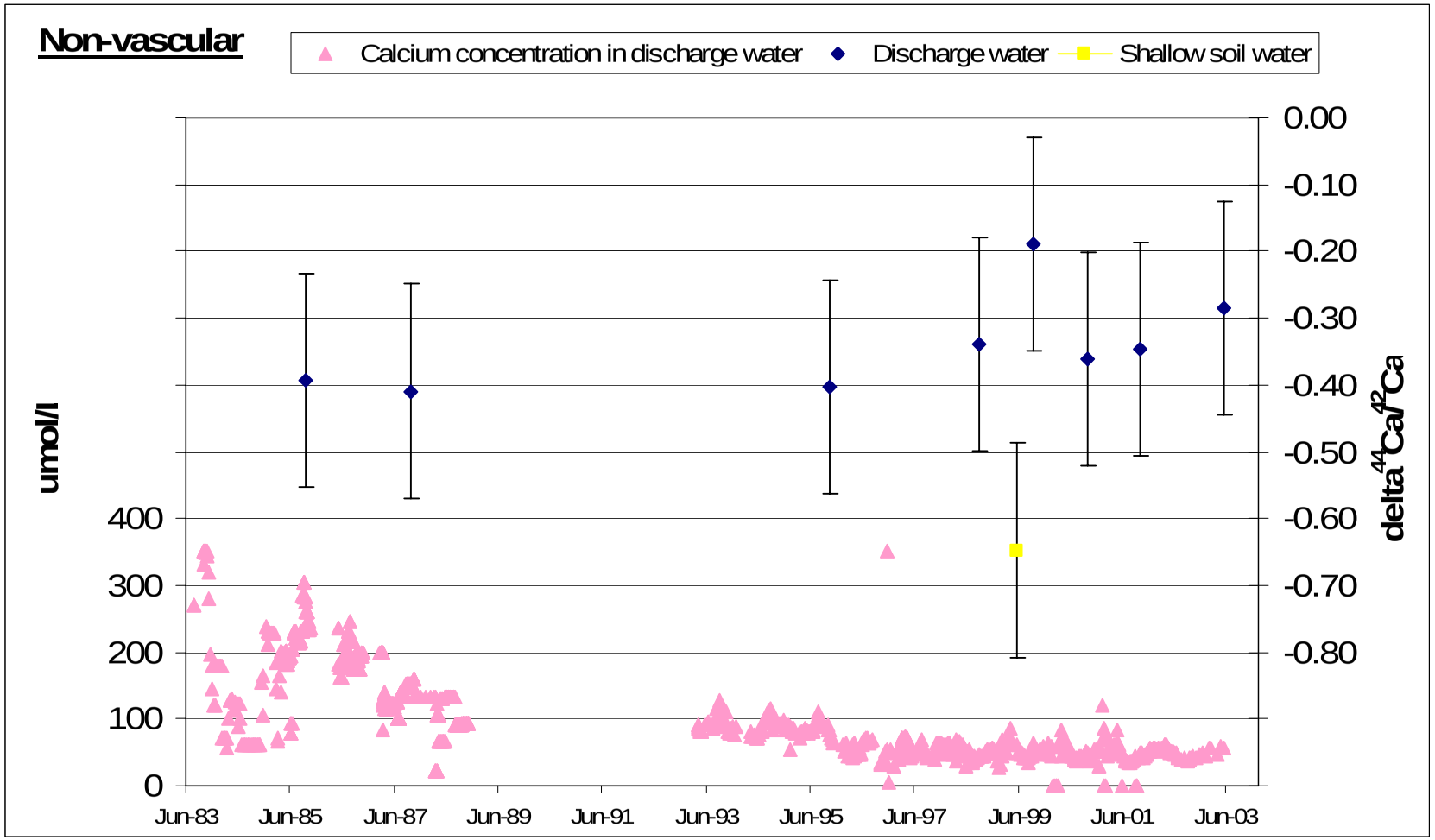


Figure 3: Calcium concentration and $\delta^{42}Ca$ of soil and discharge water in the non-vascular sandbox over the length of the experiment (±0.16 ‰) (App. C) (Balogh, 2006).

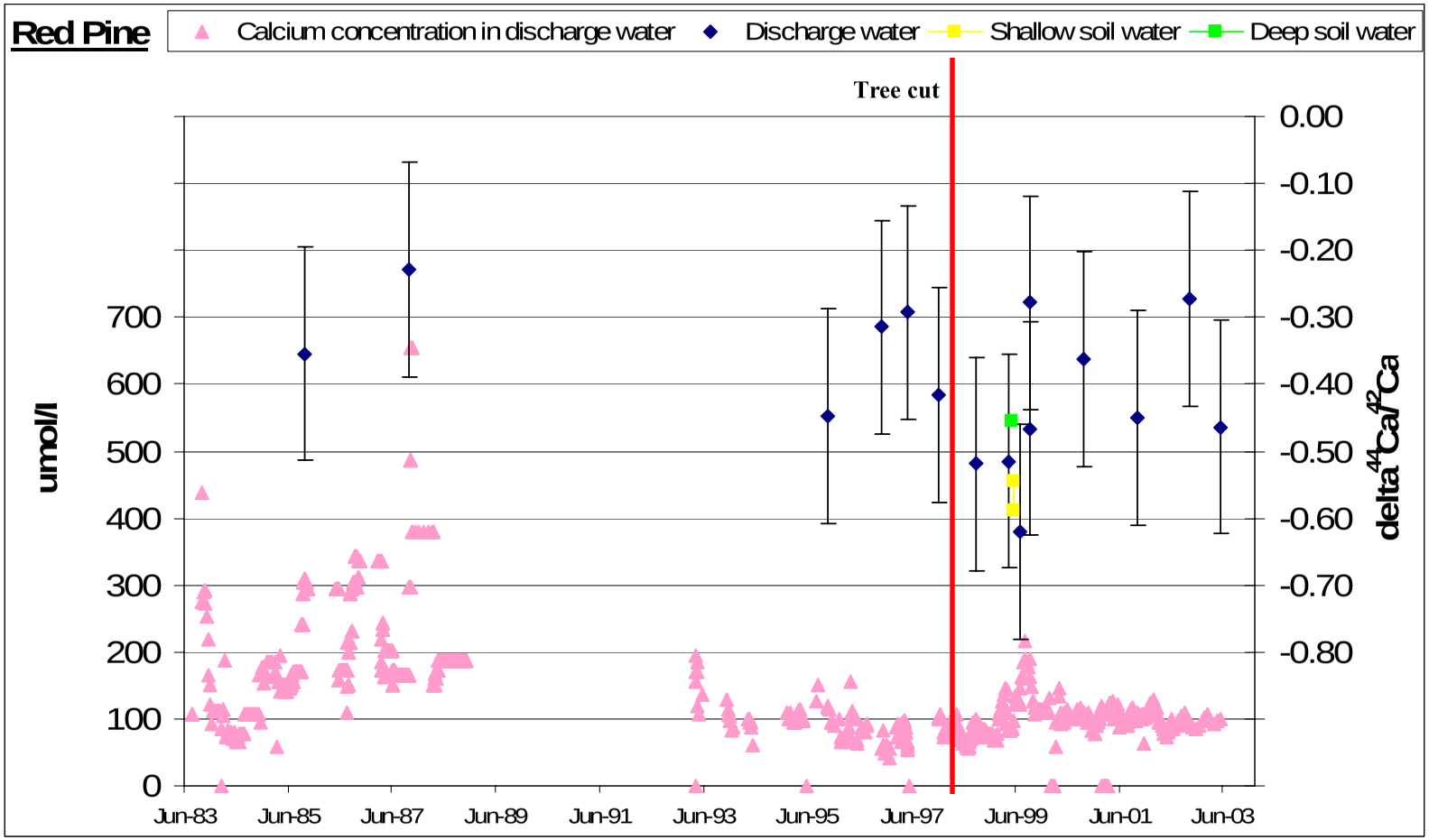


Figure 4: Calcium concentration and $\delta^{42}Ca$ of soil and discharge water in the red pine sandbox over the length of the experiment (±0.16 ‰) (App. C) (Balogh, 2006).

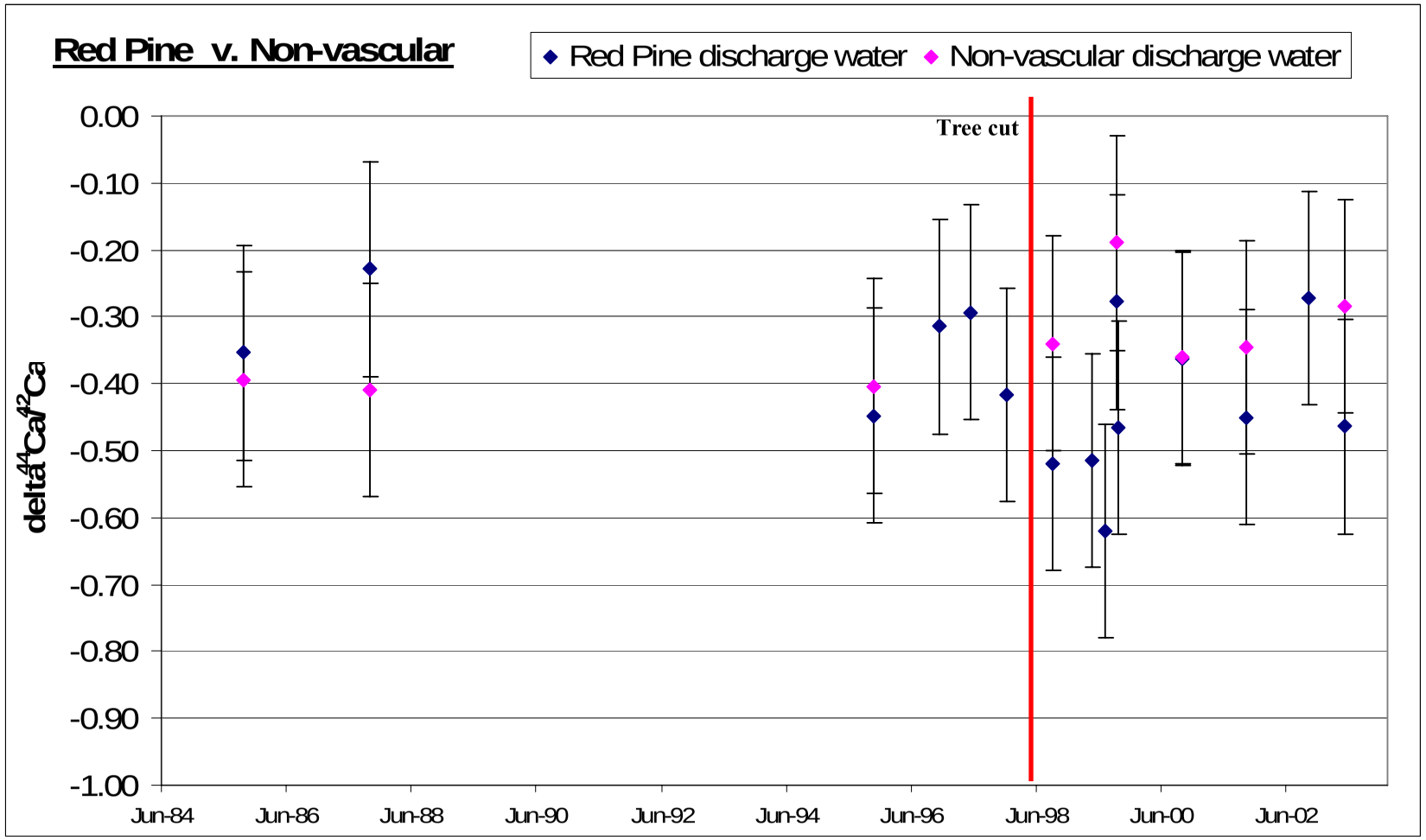


Figure 5: Comparison of $\delta^{42}Ca$ in discharge water from the red pine and non-vascular sandboxes (±0.16 ‰) (App. C)

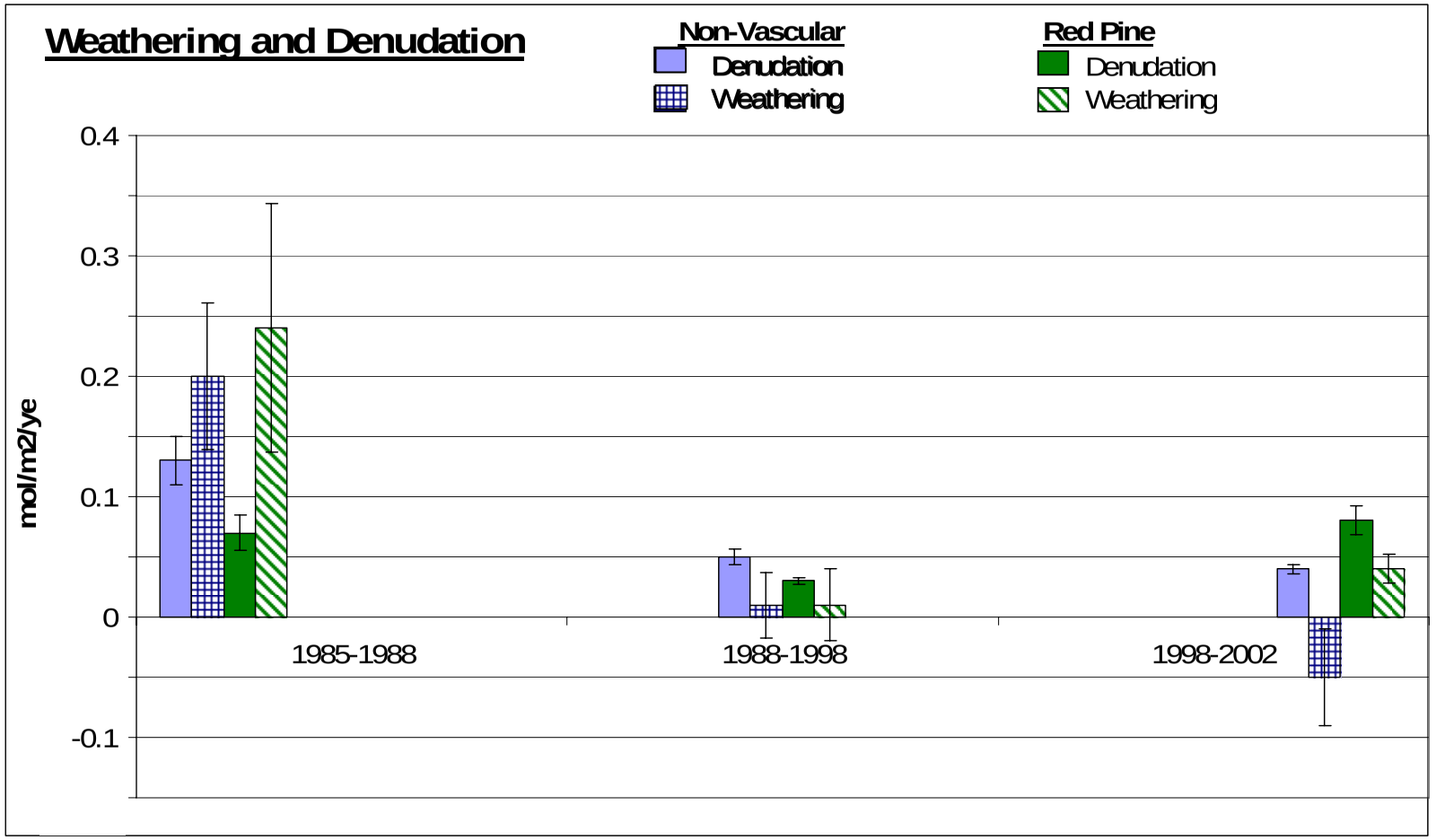


Figure 6: Weathering and denudation fluxes of calcium in the sandboxes (Balogh, 2006).

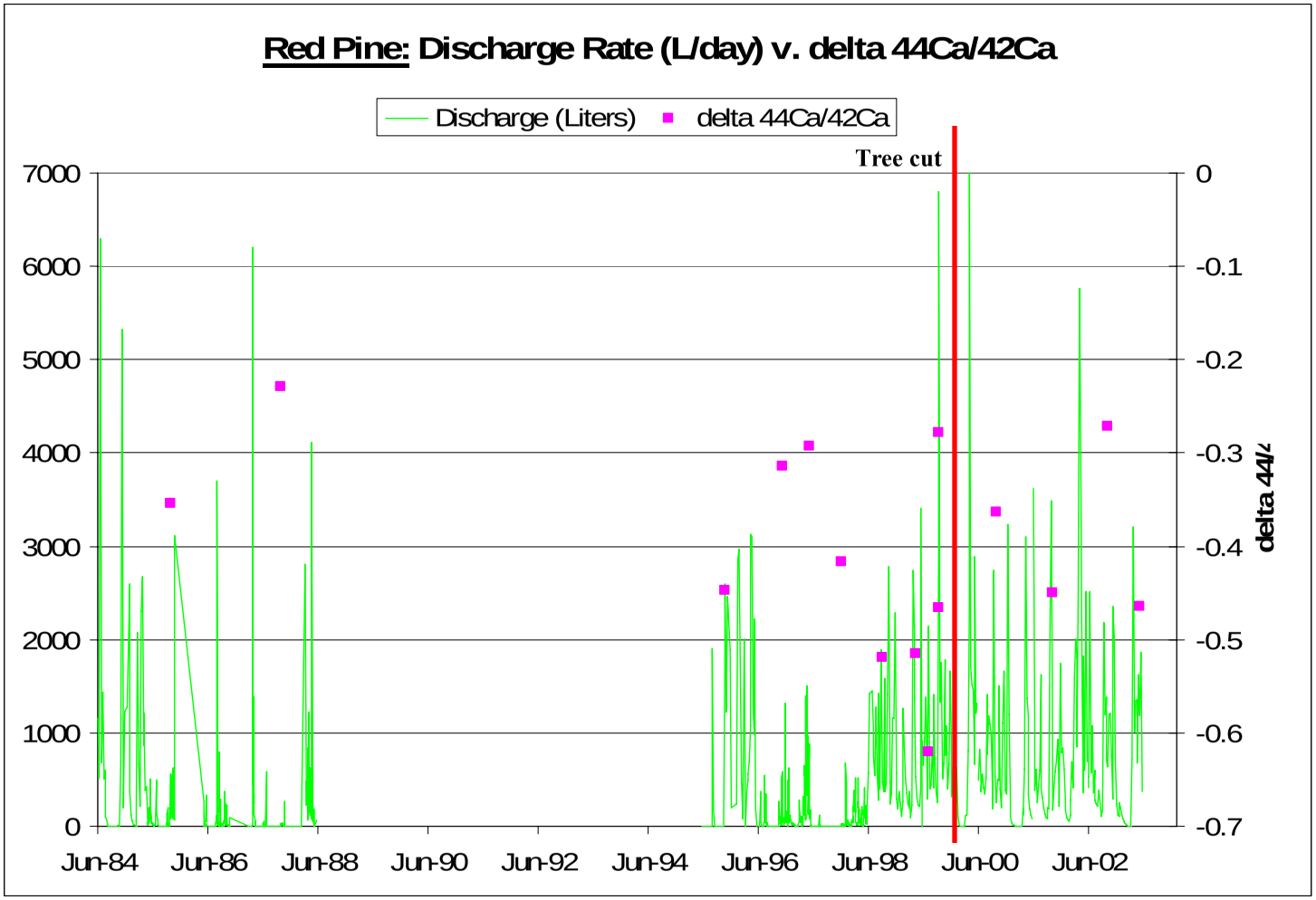


Figure 7: Discharge versus the δ42Ca value of discharge water in the red pine sandbox. Discharge data is from O'Brien (2000) and Balogh (2006).

References

Balogh, Z., 2006. Chemical Hydrology of Vascular Plant Growth: Role of root-fungus associations, PhD Dissertation, Washington State University, Pullman, WA.

Berner, R.A., Rao, J., Chang, C., O'Brien, R. and Keller, C.K., 1998. Seasonal Variability of Adsorption and Exchange Equilibria in Soil Waters. Aquatic Geochemistry, 4: 273-290.

Bormann, B.T. et al., 1993. Rapid N2 Fixation in Pines, Alders, and Locust: Evidence from the sandbox ecosystem study. Ecology, 74(2): 583-598.

Bormann, B.T., Keller, C.K., Wang, D. and Bormann, F.H., 2002. Lessons from the Sandbox: Is Unexplained Nitrogen Real? Ecosystems, 5: 727-733.

Bormann, B.T. et al., 1998. Rapid, plant-induced weathering in an aggrading experimental ecosystem. Biogeochemistry, 43: 129-155.

Bormann, F.H. et al., 1987a. The Hubbard Brook sandbox experiment. Restoration Ecology. Cambridge University Press, England, 251-256 pp.

Bormann, F.H. et al., 1987b. Restoration Ecology. The Hubbard Brook sandbox experiment. Cambridge University Press, 251-256 pp.

Bormann, F.H. and Likens, G.E., 1967. Nutrient Cycling. Science, 155: 424-429.

Bullen, T.D., Fitzpatrick, J.A., White, A.F., Schulz, M.S. and Vivit, D.V., 2004. Calcium stable isotope evidence for three soil calcium pools at a granitoid chronosequence. In: W.S. II (Editor), Water-Rock Interaction. Taylor & Francis Group, London, pp. 813-817.

Chapin III, F.S., Matson, P.A. and Mooney, H.A., 2002. Principles of Terrestrial Ecosystem Ecology. Springer Science, New York, NY, 436 pp.

DePaolo, D.J., 2004. Calcium Isotopic Variations Produced by Biological, Kinetic, Radiogenic and Nucleosynthetic Processes. Reviews in Mineralogy & Geochemistry, 55: 255-288.

Driscoll, C.T. et al., 2001. Acidic Deposition in the Northeastern United States: Sources and Inputs, Ecosystem Effects, and Management Strategies. BioScience, 51(3): 180-198.

Eisenhauer, A. et al., 2004. Proposal for International Agreement on Ca Notation Resulting from Discussions at Workshops on Stable Isotope Measurements Held in Davos (Goldschmidt 2002) and Nice (EGS-AGU-EUG 2003). Geostandards Newsletter: The Journal of Geostandards and Geoanalysis, 28(1): 149-151.

Fietzke, J. et al., 2004. Direct measurement of 44Ca/40Ca ratios by MC-ICP-MS using the cool plasma technique. Chemical Geology, 206: 11-20.

Halicz, L., Galy, A., Belshaw, N.S. and O'Nions, R.K., 1999. High-precision measurement of calcium isotopes in carbonates and related materials by multiple collector inductively coupled plasma mass spectrometry (MC-ICP-MS). Journal of Analytical Atomic Spectrometry, 14: 1835-1838.

Havig, J.R., 2002. Effects of Perturbation on Nutrient Pools and Loss from an Experimental Forest Mesocosm, Masters Thesis, Washington State University, Pullman, WA, 142 pp.

Hippler, D. et al., 2003. Calcium Isotopic Composition of Various Reference Materials and Seawater. Geostandards Newsletter: The Journal of Geostandards and Geoanalysis, 27(1): 13-19.

Ingersoll, R.C., Bormann, F.H., Bowden, W.B., Hamburg, S.P. and Bowden, R.D., 1987. The sandbox experiment notebook. Rubenstein School of Environment and Natural Resources, University of Vermont, Burlington, VT.

Keller, C.K. et al., 2006. Tree harvest in an experimental sand ecosystem: Plant effects on nutrient dynamics and solute generation. Ecosystems, 9: 634-646.

Keller, C.K., White, T.M., O'Brien, R. and Smith, J.L., in press. Soil CO2 Dynamics and fluxes as affected by tree harvest in an experimental sand ecosystem. JGR-Biogeosciences.

Likens, G.E., 2004. Some Perspectives of Long-term Biogeochemical Research from the Hubbard Brook Ecosystem Study. Ecology, 85(9): 2355-2362.

Likens, G.E. et al., 1998. The biogeochemistry of calcium at Hubbard Brook. Biogeochemistry, 41: 89-173.

McLaughlin, S.B. and Wimmer, R., 1999. Tansley Review No.104: Calcium physiology and terrestrial ecosystem processes. New Phytology, 142(373-417).

Moulton, K.L. and Berner, R.A., 1998. Quantification of the effect of plants on weathering: Studies in Iceland. Geology, 26(10): 895-898.

O'Brien, R., 2000. Unsaturated Flow and Hydrogeochemistry beneath Different Plant Covers: Field Observations from The Hubbard Brook Sandbox Experiment, PhD Dissertation, Washington State University, Pullman, WA, 255 pp.

O'Brien, R., Keller, C.K. and Strobridge, D.M., 2004. Plant-cover effects on hydrology and pedogenesis in a sandy vadose zone. Geoderma, 118: 63-76.

Perakis, S.S. et al., 2006. Coupled Nitrogen and Calcium cycles in Forests of the Oregon Coast Range. Ecosystems, 9: 63-74.

Platzner, I. and Degani, N., 1990. Fractionation of Stable Calcium Isotopes in Tissues of Date Palm Trees. Biomedical and Environmental Mass Spectrometry, 19: 822-824.

Rosman, K.J.R. and Taylor, P.D.P., 1998. Isotopic Composition of the Elements 1997. Pure & Applied Chemistry, 70(1): 217-235.

Russell, W.A. and Papanastassiou, D.A., 1978. Calcium Isotope Fractionation in Ion-Exchange Chromatography. Analytical Chemistry, 50(8): 1151-1154.

Schaberg, P.G., DeHayes, D.H. and Hawley, G.J., 2001. Anthropogenic Calcium Depletion: A Unique Threat to Forest Ecosystem Health? Ecosystem Health, 7(4): 214-228.

Schaetzl, R. and Anderson, S., 2005. Soils: Genesis and Geomorphology. Cambridge University Press, New York, USA.

Schmitt, A., Bracke, G., Stille, P. and Kiefel, B., 2001. The Calcium Isotope Composition of Modern Seawater Determined by Thermal Ionisation Mass Spectrometry. Geostandards Newsletter: The Journal of Geostandards and Geoanalysis, 25: 267-275.

Schmitt, A., Chabaux, F. and Stille, P., 2003. The calcium riverine and hydrothermal isotopic fluxes and the oceanic calcium mass balance. Earth and Planetary Science Letters, 6731: 1-16.

Skulan, J. and DePaolo, D.J., 1999. Calcium isotope fractionation between soft and mineralized tissues as a monitor of calcium use in vertebrates. Proceedings of the National Academy of Sciences of the United States of America, 96(24): 13709-13713.

Skulan, J., DePaolo, D.J. and Owens, T.L., 1997. Biological control of calcium isotopic abundances in the global calcium cycle. Geochimica et Cosmochimica Acta, 61(12): 2505-2510.

Tera, F., Eugster, O., Burnett, D.S. and Wasserburg, G.J., 1970. Comparative study of Li, Na, K, Rb, Cs, Ca, Sr and Ba abundances in achondrites and in Apollo 11 lunar samples. Proceedings of the Apollo 11 Lunar Science Conference, 2: 1637-1657.

White, W.M., Albarede, F. and Telouk, P., 2000. High-precision analysis of Pb isotope ratios by multi-collector ICP-MS. Chemical Geology, 167: 257-270.

Wieser, M.E., Buhl, D., Bouman, C. and Schwieters, J., 2004. High Precision calcium isotope ratio measurements using a magnetic sector multiple collector inductively coupled plasma mass spectrometer. Journal of Analytical Atomic Spectrometry, 19: 844-851.

Zhu, P. and MacDougall, J.D., 1998. Calcium isotopes in the marine environment and the ocean calcium cycle. Geochimica et Cosmochimica Acta, 62(10): 1691-1698.

APPENDIX A

FULL PROCEDURE FOR THE CHROMATOGRAPHIC

SEPARATION OF CALCIUM

First Stage Separation

Sample Preparation

1. Dry down filtered water sample in a savillex container (amt depends on concentration needed)
    i. –Seawater: add a few drops of 14M nitric acid after initial dry down and dry down again
2. Re-dissolve sample in 1 mL 2M HCL (titrated) and flux on hotplate for 2-3 hours
3. Sonicate 10 minutes
4. Transfer sample to centrifuge tube and centrifuge 10 minutes
5. Sample is now ready for columns

Column Preparation

1. use glass columns loaded with Dowex 50W-X8 resin
2. Backwash resin using 2M HCL and let resettle
3. You may have to do this twice to get no air bubbles

Column Specifics

- AG 50W-X8
- 8.9 mL resin
- ~17.5cm x ~1cm columns

Column Procedure

1. Load 1 mL sample
2. Wash 38 mL 2M HCl (waste); 1mL, 1mL, 1mL, 35mL
3. Elute Ca with 29 mL 2M HCl in 30mL savillex container (calcium)
4. Fill reservoir up 3 times with ~6M HCl to clean columns.

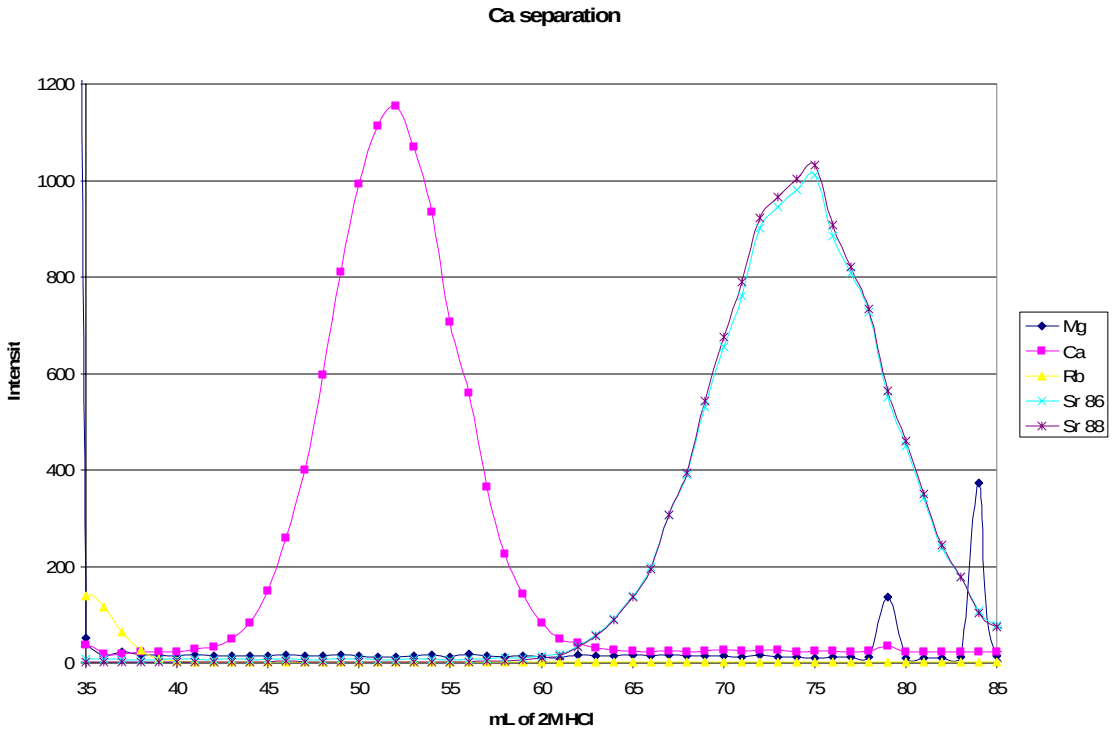


Figure A-1: Calcium elution curve, first stage separation

Second Stage Separation

Sample Preparation 2

1. Dry down 29 mL Ca sample
2. Add 2-3 drops 14 M HNO_3 and dry down
3. Add 2 ml of 8 M HNO_3
4. Cap and flux on hot plate for 2-3 hours
5. Dry down
6. Re-dissolve in 0.4 ml 8M HNO_3
7. Sample is now ready for columns

Sr-spec column preparation

1. Remove old resin from column (columns should be stored empty in HNO_3 bath)
2. Add H_2O to column to establish flow
3. Add resin slurry with pipette to start of the "flare"
4. Rinse with 1/2 reservoir of H_2O to settle resin
5. Rinse with 1/2 reservoir of 3M HNO_3
6. Rinse with 1/2 reservoir H_2O
7. Rinse with 1/2 reservoir of 8M HNO_3

Sr-spec column Procedure

Separation (use dropper bottles)

1. Put collection savillex container under columns BEFORE loading sample. You will be collecting ALL the steps in the following procedure
2. Load sample (0.4 mL 8 M HNO_3)
3. Wash with 0.4 ml of 3 M HNO_3 (10 drops)
4. Wash with 0.4 ml of 3 M HNO_3 (10 drops)
5. Wash with 0.4 ml of 3 M HNO_3 (10 drops)
6. Wash with 0.4 ml of 3 M HNO_3 (10 drops)
7. Wash with 0.4 ml of 3 M HNO_3 (10 drops)

note: can do this with any combination once Sr is washed onto column (e.g., 0.4+0.4+1.2,etc.) as long as total volume = 2.0 ml of 3M HNO3. (1 drop = ~0.04 mL)

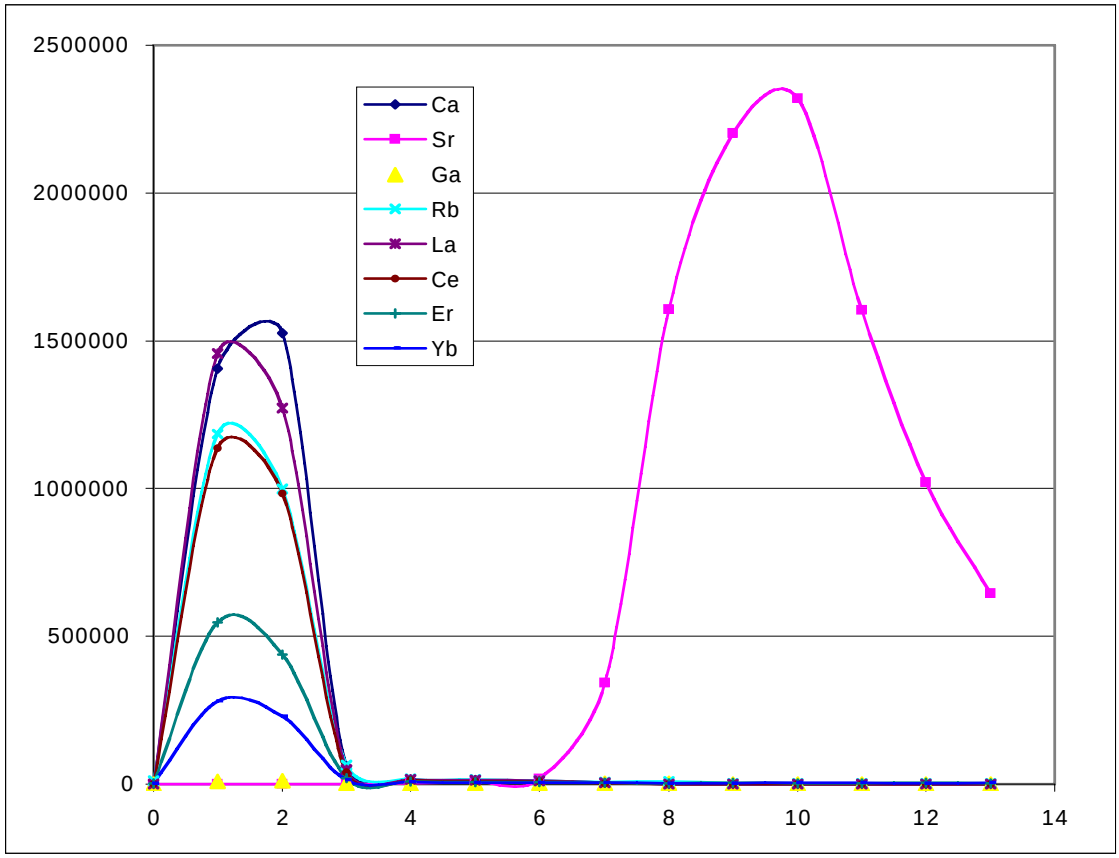


Figure A-2: Sr-spec column elution curve. Each number on x-axis represents 0.4 mL addition of acid.

Final Procedure

1. Dry down sample
2. Add a few drops of 14M HNO_3, dry down
2. Dissolve in 2% HNO_3, cap and flux in savillex container
3. Sample is now ready for Neptune

APPENDIX B

FULL PROCEDURE FOR THE DATA REDUCTION

OF CALCIUM ISOTOPE MEASUREMENTS

A) Calculate Ti-beta value for titanium mass bias

$$Ti-beta=\frac{\left(\ln\left[\frac{A}{B}\right]\right)}{\left(\ln\left[\frac{mass^{49}Ti}{mass^{47}Ti}\right]\right)}$$

Where: **A** = empirical $^{49}Ti/^{47}Ti$ ratio (taken to be **0.72715** based on natural abundances)
B = raw $^{49}Ti/^{47}Ti$ ratio measured on Neptune

B) Calculate beta-beta factor for the difference between titanium and calcium mass bias

$$beta-beta=\frac{\left(\ln\left[\frac{mass^{49}Ti}{mass^{47}Ti}\right]\right)}{\left(\ln\left[\frac{mass^{44}Ca}{mass^{42}Ca}\right]\right)}\times slope$$

Where: **slope** = slope of the line of graph of $\left(\ln\left[\frac{^{49}Ti}{^{47}Ti}\right]\right)$ plotted on the x-axis and $\left(\ln\left[\frac{^{44}Ca}{^{42}Ca}\right]\right)$ plotted on the y-axis, where the ratios are the raw (uncorrected) values

C) Calculate Ca-beta value for calcium mass bias

$$Ca-beta=(Ti-beta)\times(beta-beta)=\frac{\left(\ln\left[\frac{A}{B}\right]\right)}{\left(\ln\left[\frac{mass^{44}Ca}{mass^{42}Ca}\right]\right)}\times slope$$

D) Calculate fractionation factor (f); the correction factor for calcium mass bias

$$f=\left(\frac{mass^{44}Ca}{mass^{42}Ca}\right)^{(Ca-beta)}$$

E) Calculate mass bias corrected ratio, $\left(\frac{^{44}Ca}{^{42}Ca}\right)_{corrected}$

$$\left(\frac{^{44}Ca}{^{42}Ca}\right)_{corrected} = f \times \left(\frac{^{44}Ca}{^{42}Ca}\right)_{raw}$$

F) Calculate Daily average normalizer (D_n), a constant which normalizes data to the known value of the standard

$$D_n = \left(\frac{S}{Y}\right)$$

Where: **S** = accepted standard value for the $\left(\frac{^{44}Ca}{^{42}Ca}\right)$ ratio. (For the standard you are doing your bracketing with specifically)

Y = Average of $\left(\frac{^{44}Ca}{^{42}Ca}\right)_{corrected}$ values for your standard for the day

G) Calculate the final, correct value for sample

$$\left(\frac{^{44}Ca}{^{42}Ca}\right)_{Sample} = (D_n) \times \left(\frac{^{44}Ca}{^{42}Ca}\right)_{corrected}$$

H) Calculate δ^{42}Ca

$$\delta^{42}Ca = \left(\left[\frac{\left(\frac{^{44}Ca}{^{42}Ca}\right)_{sample}}{\left(\frac{^{44}Ca}{^{42}Ca}\right)_{seawater}}\right] - 1\right) * 1000$$

Where: $\left(\frac{44Ca}{42Ca}\right)_{seawater} = 3.2719$

APPENDIX C

CALCIUM CONCENTRATIONS AND $\delta^{42}Ca$ OF SOIL AND DISCHARGE WATER IN THE NON-VASCULAR AND RED PINE SANDBOXES

Discharge water

Sample Name	Date	$^{44}Ca/^{42}Ca$ (corrected)*	$\delta^{42}Ca$ (‰)	Ca concentration (ppm)
RP-1-W	10/1/1985	3.2707	-0.35	12.4
RP-2-W	10/8/1987	3.2711	-0.23	11.9
RP-3-W	10/26/1995	3.2704	-0.45	4.8
RP-4-W	11/11/1996	3.2708	-0.31	2.2
-	4/8/1997	-	-	3.7
RP-6-W	5/13/1997	3.2709	-0.29	2.2
RP-7-W	12/9/1997	3.2705	-0.42	4.0
RP-8-W	9/8/1998	3.2702	-0.52	4.0
RP-9-W	4/20/1999	3.2702	-0.51	3.4
-	5/11/1999	-	-	3.5
-	6/15/1999	-	-	5.2
RP-12-W	7/13/1999	3.2698	-0.62	5.8
-	8/17/1999	-	-	7.7
RP-14-W	9/14/1999	3.2709	-0.28	7.6
RP-15-W	9/21/1999	3.2703	-0.47	6.6
-	10/19/1999	-	-	4.7
RP-17-W	10/3/2000	3.2707	-0.36	4.4
RP-18-W	10/9/2001	3.2704	-0.45	4.2
RP-19-W	10/15/2002	3.2710	-0.27	3.8
RP-20-W	5/13/2003	3.2703	-0.46	4.0
NV-1-W	10/1/1985	3.2706	-0.39	10.4
NV-2-W	10/8/1987	3.2705	-0.41	6.1
NV-3-W	10/26/1995	3.2705	-0.40	3.1
-	12/9/1997	-	-	2.5
NV-5-W	9/8/1998	3.2707	-0.34	1.8
NV-6-W	9/14/1999	3.2712	-0.19	2.4
NV-7-W	10/3/2000	3.2707	-0.36	2.0
NV-8-W	10/9/2001	3.2707	-0.35	1.9
-	10/15/2002	-	-	1.8
NV-10-W	5/13/2003	3.2709	-0.28	2.2

*for uncorrected isotope values see Appendix D

Soil Water

Sample Name	Date	Depth (cm)	Sampler	$^{44}Ca/^{42}Ca$ (corrected)*	$\delta^{42}Ca$ (‰)	Ca concentration (ppm)
RP-1-SW	5/9/1999	15	RP1-BF	3.2697	-0.65	1.6
RP-2-SW	5/9/1999	95	RP3-BF	3.2702	-0.51	9.3
RP-3-SW	5/21/1999	15	RP1-AF	3.2704	-0.46	3.7
RP-4-SW	5/21/1999	15	RP1-BF	3.2701	-0.55	11.0
NV-1-SW	5/21/1999	15	NV1-BF	3.2699	-0.59	7.3

*for uncorrected isotope values see Appendix D

APPENDIX D

UNCORRECTED ISOTOPE RATIOS AS RECEIVED FROM

ANALYSIS ON THE MC-ICP-MS

January 24, 2006

Sample Name	run	$^{49}Ti/^{47}Ti$	$^{43}Ca/^{42}Ca$	$^{44}Ca/^{42}Ca$
Ca-Ti std.1	1	0.81933350	0.21899114	3.5579461
Ca-Ti std.2	2	0.81954063	0.21903060	3.5586937
Ca-Ti std.3	3	0.81969897	0.21904210	3.5591699
Seawater.1	4	0.81962371	0.21899389	3.5602904
Ca-Ti std.4	5	0.81979108	0.21903804	3.5591780
Seawater.2	6	0.81983450	0.21902742	3.5613578
Ca-Ti std.5	7	0.81979307	0.21900719	3.5589865
Seawater.3	8	0.81983756	0.21904388	3.5606514
Ca-Ti std.6	9	0.81987823	0.21907980	3.5582809
Seawater.4	10	0.81990627	0.21905106	3.5609369
Ca-Ti std.7	11	0.82004600	0.21912172	3.5588669
Seawater.5	12	0.81996216	0.21904197	3.5610462
Ca-Ti std.8	13	0.82012250	0.21910131	3.5586718
Seawater.6	14	0.81998665	0.21906092	3.5613488
Ca-Ti std.9	15	0.82000371	0.21909487	3.5587625
Seawater.7	16	0.81998835	0.21907571	3.5613135
Ca-Ti std.10	17	0.82007705	0.21910598	3.5588258
Seawater.8	18	0.82014069	0.21906809	3.5616969
Ca-Ti std.11	19	0.82007169	0.21912465	3.5592950
Ca-Ti std.12	20	0.82020276	0.21911791	3.5595739

January 31, 2006

Sample Name	run	$^{49}Ti/^{47}Ti$	$^{43}Ca/^{42}Ca$	$^{44}Ca/^{42}Ca$
Ca-Ti std.1	1	0.81895746	0.21910146	3.5576626
Ca-Ti std.2	2	0.81909067	0.21912564	3.5580274
Ca-Ti std.3	3	0.81930375	0.21912096	3.5588050
Ca-Ti std.4	4	0.81953591	0.21914756	3.5599116
Ca-Ti std.5	5	0.81965733	0.21914460	3.5601078
Seawater.1	6	0.81979881	0.21912711	3.5632701
Ca-Ti std.6	7	0.81994362	0.21917010	3.5611951
Ca-Ti std.7	8	0.81999235	0.21918590	3.5615048
RP-1-W.1	9	0.82021872	0.21911504	3.5634814
Ca-Ti std.8	10	0.82025603	0.21921750	3.5626881
RP-2-W.1	11	0.82057848	0.21924383	3.5651282
Ca-Ti std.9	12	0.82043929	0.21924222	3.5634012
Seawater.2	13	0.82051845	0.21922141	3.5664203
Ca-Ti std.10	14	0.82055861	0.21925269	3.5636842
Ca-Ti std.11	15	0.82068777	0.21927233	3.5644712
Seawater.3	16	0.82063552	0.21923937	3.5668315
Ca-Ti std.12	17	0.82060706	0.21927097	3.5639895

February 24, 2006

Sample Name	run	$^{49}Ti/^{47}Ti$	$^{43}Ca/^{42}Ca$	$^{44}Ca/^{42}Ca$
Ca-Ti std.1	1	0.81546654	0.21867170	3.5432713
Ca-Ti std.2	2	0.81552240	0.21869713	3.5436575
Ca-Ti std.3	3	0.81554804	0.21868361	3.5436037
Ca-Ti std.4	4	0.81563160	0.21868195	3.5440239
Seawater.1	5	0.81570285	0.21862181	3.5460698
Ca-Ti std.5	6	0.81568882	0.21869966	3.5439175
Ca-Ti std.6	7	0.81575978	0.21872796	3.5442768
NV-1-W.1	8	0.81592887	0.21858476	3.5456805
Ca-Ti std.7	9	0.81591928	0.21871967	3.5449187
RP-15-W.1	10	0.81604685	0.21857694	3.5458759
Ca-Ti std.8	11	0.81614711	0.21874991	3.5456853
RP-6-W.1	12	0.81621390	0.21861722	3.5471301
Ca-Ti std.9	13	0.81625784	0.21877629	3.5461232
RP-17-W.1	14	0.81639793	0.21865838	3.5475875
Ca-Ti std.10	15	0.81639268	0.21879454	3.5467825
NV-6-W.1	16	0.81637641	0.21869223	3.5481208
Ca-Ti std.11	17	0.81654995	0.21881233	3.5474417
RP-4-W.1	18	0.81665622	0.21870359	3.5487480
Ca-Ti std.12	19	0.81654238	0.21880626	3.5474142
NV-2-W.1	20	0.81659035	0.21867740	3.5481603
Ca-Ti std.13	21	0.81664032	0.21882030	3.5478984
RP-14-W.1	22	0.81671881	0.21870843	3.5491179
Ca-Ti std.14	23	0.81674224	0.21881010	3.5480656
NV-10-W.1	24	0.81679033	0.21868664	3.5493698
Ca-Ti std.15	25	0.81683568	0.21882495	3.5484252
RP-19-W.1	26	0.81692950	0.21872174	3.5499465
Ca-Ti std.16	27	0.81692422	0.21885958	3.5490467
NV-3-W.1	28	0.81693268	0.21872308	3.5498846
Ca-Ti std.17	29	0.81690950	0.21884654	3.5486510
RP-2-W.2	30	0.81704482	0.21877714	3.5505434
Ca-Ti std.18	31	0.81698819	0.21888314	3.5492480
RP-1-W.2	32	0.81708844	0.21873689	3.5500554
Ca-Ti std.19	33	0.81703825	0.21887266	3.5492406
Seawater.2	34	0.81711596	0.21879452	3.5516272
Ca-Ti std.20	35	0.81712987	0.21887406	3.5495231

March 8, 2006

Sample Name	run	$^{49}Ti/^{47}Ti$	$^{43}Ca/^{42}Ca$	$^{44}Ca/^{42}Ca$
Ca-Ti std.1	1	0.81285196	0.21818106	3.5324986
Ca-Ti std.1	2	0.81278489	0.21819421	3.5324298

Ca-Ti std.1	3	0.81309400	0.21829362	3.5343208
Ca-Ti std.1	4	0.81327046	0.21829622	3.5346875
Seawater.1	5	0.81334243	0.21823206	3.5376466
Ca-Ti std.1	6	0.81333465	0.21832329	3.5353811
Seawater.2	7	0.81337276	0.21824473	3.5375035
Ca-Ti std.1	8	0.81338960	0.21847659	3.5374713
Seawater.3	9	0.81343430	0.21837074	3.5394323
Ca-Ti std.1	10	0.81342478	0.21846562	3.5371338
Seawater.4	11	0.81344024	0.21836786	3.5391117
Ca-Ti std.1	12	0.81340070	0.21845977	3.5368858
Seawater.5	13	0.81347376	0.21837276	3.5395999

March 10, 2006

Sample Name	**run**	**$^{49}Ti/^{47}Ti$**	**$^{43}Ca/^{42}Ca$**	**$^{44}Ca/^{42}Ca$**
Ca-Ti std.1	1	0.81217456	0.21832731	3.5293083
Ca-Ti std.2	2	0.81224490	0.21832928	3.5296408
Ca-Ti std.3	3	0.81235734	0.21833699	3.5300941
Ca-Ti std.4	4	0.81246235	0.21834555	3.5305443
Seawater.1	5	0.81260032	0.21824558	3.5331130
Ca-Ti std.5	6	0.81269998	0.21837358	3.5314808
Ca-Ti std.7	8	0.81297970	0.21838386	3.5318924
NV-8-W.1	9	0.81311668	0.21824280	3.5330703
Ca-Ti std.8	10	0.81312033	0.21839228	3.5325894
RP-3-W.1	11	0.81318961	0.21825029	3.5331194
Ca-Ti std.9	12	0.81312867	0.21840311	3.5327078
NV-5-W.1	13	0.81315827	0.21828406	3.5337523
Ca-Ti std.10	14	0.81309905	0.21841914	3.5330924
RP-9-W.1	15	0.81322931	0.21825775	3.5334678
Ca-Ti std.11	16	0.81321718	0.21842425	3.5333769
RP-7-W.1	17	0.81319373	0.21826307	3.5337921
Ca-Ti std.12	18	0.81320554	0.21841904	3.5330450
RP-12-W.1	19	0.81317095	0.21824956	3.5327698
Ca-Ti std.13	20	0.81321274	0.21840808	3.5327739
Ca-Ti std.14	21	0.81310484	0.21841111	3.5327737
RP-18-W.1	22	0.81312356	0.21825627	3.5331776
Ca-Ti std.15	23	0.81304295	0.21839774	3.5326592
RP-20-W.1	24	0.81308831	0.21825138	3.5331262
Ca-Ti std.16	25	0.81306245	0.21838899	3.5327676
Seawater.2	26	0.81301264	0.21828562	3.5348820
Ca-Ti std.17	27	0.81303855	0.21839960	3.5326395
NV-7-W.1	28	0.81312760	0.21824958	3.5335590
Ca-Ti std.18	29	0.81306410	0.21841308	3.5329228
RP-8-W.1	30	0.81307712	0.21824969	3.5333315

Ca-Ti std.19	31	0.81302280	0.21842007	3.5333056

April 18, 2006

Sample Name	run	$^{49}Ti/^{47}Ti$	$^{43}Ca/^{42}Ca$	$^{44}Ca/^{42}Ca$
Ca-Ti std.1	1	0.80762126	0.21747195	3.5078791
Ca-Ti std.1	2	0.80759797	0.21747259	3.5079272
Ca-Ti std.1	3	0.80757580	0.21746634	3.5078372
Seawater.1	4	0.80757033	0.21732500	3.5097541
Ca-Ti std.1	5	0.80760625	0.21747226	3.5078202
RP-2-SW.1	6	0.80771431	0.21728687	3.5083506
Ca-Ti std.1	7	0.80765101	0.21748396	3.5080022
RP-1-SW.1	8	0.80767174	0.21728628	3.5082373

May 2, 2006

Sample Name	run	$^{49}Ti/^{47}Ti$	$^{43}Ca/^{42}Ca$	$^{44}Ca/^{42}Ca$
Ca-Ti std.1	1	0.80702530	0.21749660	3.5073758
Ca-Ti std.1	2	0.80703744	0.21750477	3.5073509
Ca-Ti std.1	3	0.80689216	0.21747991	3.5067813
Seawater.1	4	0.80692257	0.21734330	3.5085021
Ca-Ti std.1	5	0.80684916	0.21748054	3.5063875
RP-4-SW.1	6	0.80680295	0.21728303	3.5064202
Ca-Ti std.1	7	0.80681990	0.21749371	3.5065145
RP-3-SW.1	8	0.80680222	0.21729917	3.5068062
Ca-Ti std.1	9	0.80679682	0.21751177	3.5066372
NV-1-SW.1	10	0.80694587	0.21729096	3.5063490
Ca-Ti std.1	11	0.80686310	0.21750180	3.5065293
Seawater.2	12	0.80690913	0.21737416	3.5086660
Ca-Ti std.1	13	0.80692378	0.21749938	3.5066442

May 3, 2006

Sample Name	run	$^{49}Ti/^{47}Ti$	$^{43}Ca/^{42}Ca$	$^{44}Ca/^{42}Ca$
Ca-Ti std.1	1	0.80741923	0.21752428	3.5074262
Ca-Ti std.2	2	0.80735311	0.21750951	3.5072404
Seawater.1	3	0.80737114	0.21736449	3.5091127
Ca-Ti std.3	4	0.80735933	0.21749613	3.5070672
RP-3-SW.2	5	0.80732014	0.21729309	3.5070345
Ca-Ti std.4	6	0.80732099	0.21749978	3.5069660

May 4, 2006

Sample Name	run	$^{49}Ti/^{47}Ti$	$^{43}Ca/^{42}Ca$	$^{44}Ca/^{42}Ca$
Ca std.1	1	0.69332520	0.21777349	3.5145239
Ca std.2	2	0.69586238	0.21778915	3.5148645
Seawater.noTi.1	3	0.71011864	0.21766038	3.5170447
Ca std.3	4	0.69414597	0.21779638	3.5152322
Seawater.noTi.2	5	0.70524978	0.21766722	3.5174889
Ca.std.4	6	0.66277386	0.21780200	3.5156698
Ca-Ti std.1	7	0.80830648	0.21766538	3.5115280
NV-3-W.2	8	0.80827303	0.21751835	3.5117317
Ca-Ti std.2	9	0.80827447	0.21767148	3.5114703
Seawater.1	10	0.80824132	0.21753564	3.5133930
Ca-Ti std.3	11	0.80824173	0.21766776	3.5113636

APPENDIX E

CONTRIBUTION OF BIOMASS TO THE CALCIUM CONCENTRATION OBSERVED IN THE 1999 WATER YEAR IN THE RED PINE SANDBOX

Biomass (red pine sandbox, May 1998 (Havig, 2002)

Fine roots + Large roots + root collar = 11000 kg/ha
= 62 kg in sandbox

Litter = 32915 kg/ha
= 185 kg in sandbox

Carbon Content (Havig, 2002)

Fine roots + Large roots + root collar = 730000 mol/ha

Litter = 1320000 mol/ha

Carbon in Biomass

Fine roots + Large roots + root collar = 66.36 moles of C / kg of roots

Litter = 40.10 moles of C / kg of roots

Amount of Carbon Discharged in the 1999 water year (June 1, 1999 – June 1, 2000) (Keller et al., in press)

481 (±151) g C m^{-2} yr^{-1}

2254.688 moles of Carbon

Calcium Content (Havig, 2002)

Fine roots + Large roots + root collar = 343 mol/ha

Litter = 3752 mol/ha

Calcium in Biomass

Fine roots + Large roots + root collar = 0.03 moles of Calcium / kg of roots

Litter = 0.11 moles of Calcium / kg of roots

Amount of Calcium Discharged in the 1999 water year (June 1, 1999 – June 1, 2000) (Havig, 2002; Appendix C)

7.6883 moles of Calcium

Amount of roots or litter that decayed according to the amount of carbon discharged in 1999

= (mol C / (mol C / kg root))

34.0 kg of root or 56.2 kg of litter

How much calcium in the 1999 water year that can be attributed to the decayed roots or litter

= (kg decayed / (calcium/kg root))

Roots: 1.1 moles Ca
14 % ± 5% of calcium in 1999

Litter: 6.4 moles Ca
83 % ± 27% of calcium in 1999