Oxygen Isotope Composition of Phosphate Along Vertical Weathering Profiles that Developed on Igneous Rocks: Testing Modern Analogues for Identifying Terrestrial Life in Ancient Paleosols

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OXYGEN ISOTOPE COMPOSITION OF PHOSPHATE ALONG VERTICAL WEATHERING PROFILES THAT DEVELOPED ON IGNEOUS ROCKS: TESTING MODERN ANALOGUES FOR IDENTIFYING TERRESTRIAL LIFE IN ANCIENT PALEOSOLS

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ABSTRACT

Life on Earth began in its early oceans before moving on to land. Determining the exact timing of an established biosphere on land, however, has been elusive, due largely to the lack of either fossil or chemical evidence on land deposits. Here I test a hypothesis that the activity of land biota can be recorded in the oxygen isotope composition of phosphate in weathering profiles developed on igneous parent rocks. Igneous rock contains phosphate (PO₄³⁻), which is readily utilized for biological activities during soil development. The oxygen in PO₄³⁻ does not readily exchange its oxygen with ambient water. However, during intracellular pyrophosphate hydrolysis, the P-O bonds in PO₄³⁻ break and reform repeatedly, resulting in exchange of oxygen with that of ambient water. Since the exchange occurs at low temperature and approaches equilibrium, the biologically processed PO₄³⁻ will have much higher δ¹⁸O values than the ones from igneous sources. Thus, if an igneous weathering profile had been colonized by land biota, we shall expect to see an increase in the oxygen isotope composition of the bulk phosphate (δ¹⁸O_PO₄) from pristine unweathered igneous rocks to the upper more weathered ones. To test this hypothesis, I targeted two recent weathering profiles: 1) a basalt weathering profile with continuous weathering since ~2 Ma near the town of Karamu, New Zealand, and 2) a basalt weathering profile with continuous weathering since ~4 Ma on Hainan Island in China. Samples were collected vertically across the profiles and then phosphate was extracted from them and precipitated as silver phosphate. Phosphate analyzed from the New Zealand profile shows a dramatic increase in its δ¹⁸O across the entire weathered section relative to fresh basalt. The δ¹⁸O_PO₄ of weathered samples range from ~19.4-24.9‰ while fresh unweathered parent basalt samples are between 7.4-8.6‰. The transition between the two is abrupt.
The data from the Hainan profile are also consistent with the presence of biological P-recycling. The result of this study has an important implication for chronicling the early colonization of land by life. As long as there were biological activities on land the phosphate oxygen isotope composition will display a dramatic increase in $\delta^{18}O_{P04}$ up soil profiles developed on igneous parent rocks. Even if the upper section of an ancient paleosol is not preserved, which is often the case for paleosols, the dramatic increase in $\delta^{18}O_{P04}$ at the very beginning of signs of weathering, as shown by the Karamu section, increases the possibility of discerning early land biota activities in old weathering profiles developed on igneous rocks.
1. INTRODUCTION

1.1 Evidence for Earliest Land Biota: Body Fossils and Chemical Markers

Life on Earth began over 3.5 billion years ago (Altermann and Kazmierczak, 2003; Mojzsis et al., 1996; Noffke et al., 2013; Ohtomo et al., 2014; Schopf, 1993) in Earth’s early oceans. Exactly when it first made the transition on to land is still unknown, but it is clear that fully developed terrestrial ecosystems were in place by the late Devonian and there is fossil evidence of land plants beginning as early as the Ordovician (Gensel, 2008; Shear, 2000). Microbial life, however, has been shown to inhabit land environments much earlier with terrestrial microfossils that date back to 1.2 Ga (Horodyski and Knauth, 1994). Furthermore, some believe that their presence on land may extend back much further than indicated by the fossil record, possibly even evolving concurrently with life in the ocean (Beraldi-Campesi, 2013). This is, in part, because terrestrial environments are erosive in nature, resulting in a lack of representation in the rock record and poor conditions for fossil preservation. Metamorphism and hydrothermal alteration often affect older rocks, which add further to the difficulty of identifying life on land in Earth’s past. In addition, recent studies have been building the case that microbial life on land may have originated as early as the Archean using other biomarkers such as, stable isotopes (C, S), element ratios (C, H, N, P), and element mobility (P) (Beraldi-Campesi, 2013; Gutzmer and Beukes, 1998; Martini, 1994; Neaman et al., 2005; Rasmussen et al., 2009; Stueken et al., 2012; Watanabe et al., 2000). However these biomarkers are often ambiguous and are not widely accepted. Therefore, more convincing biomarkers are crucial to our understanding history of life on Earth, particularly when life first inhabited terrestrial environments.
1.2 Hypothesis

Any significant activity of land biota would result in an increase in the oxygen isotope composition of phosphate ($\delta^{18}O_{PO_4}$) from a fresh parent igneous rock to the more weathered samples in the upper soil profile.

1.3 Rationale: Enzyme Catalyzed PO$_4$-H$_2$O Isotope Exchange

Phosphorus is a fundamental and often limited nutrient required by all living organisms. In nature, phosphorus is almost always found as phosphate. Recently, attention has been given to the oxygen isotope composition of biologically cycled phosphate in soils and sediments; Figure 1.1 (Angert et al., 2012; Angert et al., 2011; Tamburini et al., 2010; Weiner et al., 2011; Zohar et al., 2010). On land the primary source of phosphate comes from parent materials on which a soil profile is developing and partially from wind blown dust (Mahowald et al., 2008; Okin et al., 2004; Swap et al., 1992). As the parent materials weather into soils, phosphate-bearing minerals become available for biological processing where they are dissolved and phosphate is taken up by living organisms. While there is no isotope fractionation associated with dissolution of apatite (Lecuyer et al., 1999), one laboratory study has shown that in a pool of dissolved inorganic phosphate E. coli will preferentially uptake lighter ($^{16}$O) phosphate while leaving the heavier ($^{18}$O) behind (Blake et al., 2005). However, this situation is different from the weathering of igneous rock and more research needs to be done to determine if this effect is present in other microorganisms, plants, and fungi.

Once phosphate is inside an organism it can be used in a multitude of biological applications. Some of the more common uses include formation of organic compounds like the adenosine phosphates (ATP, ADP, and AMP) and nucleotides in DNA and RNA. Enzyme
catalyzed hydrolysis of these compounds result in oxygen isotope exchange with ambient water. For example, pyrophosphate (P₂O₇), a product of ATP reactions, will inherit one oxygen atom from ambient water during its hydrolysis, via the enzyme pyrophosphatase (PPase), forming two separate phosphate ions (Harold, 1966). This process has been shown to be fast and reversible, quickly bringing PO₄³⁻ into equilibrium with ambient water (Blake et al., 2005; Cohn, 1953; Paytan et al., 2002).

\[
H_2P_2O_7^{2-} + H_2O \rightleftharpoons 2HPO_4^{2-} + 2H^+ \tag{1}
\]

In fact, PPase appears to be the dominating factor controlling the oxygen isotope composition of biologically processed phosphate (Blake et al., 2005). Further evidence of this rapid oxygen exchange with water is provided by previous observations of biogenic apatite in thermodynamic equilibrium with water (Kolodny et al., 1983; Longinelli and Nuti, 1973):

\[
\delta^{18}O_p = \delta^{18}O_{\text{Water}} + (111.4 - T)/(4.3) \tag{2}
\]

and more recently in lab experiments generating apatite from microbial processed phosphate in soils (Blake et al., 1998b; Blake et al., 1997):

\[
\delta^{18}O_p = \delta^{18}O_{\text{Water}} + (155 - T)/(6.4) \tag{3}
\]

where T is temperature in degrees Celsius and \(\delta^{18}O\) is defined as:

\[
\delta^{18}O = \left( \frac{^{18}O}{^{16}O} \right)_{\text{Sample}} - 1 \times 1000 \tag{4}
\]

Intracellular oxygen-isotopically equilibrated PO₄³⁻ can then make its way back into the soil by being expelled during cell growth as free phosphate ions (P₁) or during cell lysis as both P₁ and ester bound organic phosphate (P₀). Once outside of the cell, P₀ is hydrolyzed
Figure 1.1—Schematic depicting the phosphate cycle in the soils with known isotope fractionations. Biogenic apatite is apatite that contains biologically cycled phosphate (Angert et al., 2012; Ayliffe et al., 1992; Blake et al., 1998b; Blake et al., 2005; Blake et al., 1997; Lecuyer et al., 1999; Liang and Blake, 2006; Liang and Blake, 2007; Liang and Blake, 2009; Markel et al., 1994; von Sperber et al., 2014).

to generate $P_i$ by extracellular enzymes. $P_i$ is a form of phosphate that can be readily utilized by organisms. Unlike the intracellular enzyme pyrophosphates, the extracellular enzymes only exhibit irreversible kinetic isotope effects and there does not appear to be a dominant enzyme controlling the final isotope composition. This is, in part, because a series of enzymes (Figure 1.2) are required to break large organic compounds down into $P_i$, a process that can vary from one substrate to another and each has its own isotope effects.
Currently, fractionation factors for only a few of these enzymes have been determined with a wide range from -30 to 20‰ (Liang and Blake, 2006; Liang and Blake, 2009; von Sperber et al., 2014).

Figure 1.2—An example of phosphoesters working together in series to release phosphate from a nucleotide made of phosphates bonded to base and sugar compounds. Step one involves a molecular reorganization forming a phosphodiester and the release of the lower sugar and base compounds. This is followed with step 2, the hydrolysis of the phosphodiester incorporating one O isotope from water and forming a phosphomonoester. Step 3 is the hydrolysis of the phosphomonoester releasing a free phosphate molecule and incorporating a second oxygen isotope from water. Redrawn from Blake et al. (2005) and Nannipieri et al. (2011).

Once $P_i$ is released back into the soil solution it can be: 1) taken up by other organisms, 2) adsorbed by iron oxides (Froelich et al., 1977; Mills and Elderfield, 1995)
with negligible associated oxygen isotope fractionation (Jaisi et al., 2010), or 3) precipitate as apatite with a minor \( \sim 1\% \) oxygen isotope fractionation (Liang and Blake, 2007).

Ultimately, however, the intracellular enzyme pyrophosphatase (PPase) appears to be the dominating factor controlling the isotopic composition of biologically processed phosphate (Blake et al., 1998a; Blake et al., 2005). Therefore, since the bonds between oxygen and phosphorus in phosphate are very strong and do not exchange with water at temperatures \(<70^\circ\text{C}\) (Blake et al., 1997; Lecuyer et al., 1999; O’Neil et al., 2003; Tudge, 1960) unless catalyzed by biologically produced enzymes (Blake et al., 1997; Dahms and Boyer, 1973; Kolodny et al., 1983; Tudge, 1960), it is presumed that any deviation in the weathering profile from the \( \delta^{18}\text{O}_{\text{PO}_4} \) of the parent rock will be from biological processes.

Igneous rocks are often enriched in \( \text{PO}_4^{3-} \), mostly in the form of apatite, and they are the ultimate source of \( \text{PO}_4^{3-} \) on Earth. The \( \delta^{18}\text{O}_{\text{PO}_4} \) in basalts and granites are well clustered at \( \sim 6-8\% \) (VSMOW) (Angert et al., 2012; Ayliffe et al., 1992; Markel et al., 1994). This is because the apatite in them was originally in equilibrium with bulk silicates at high-temperature melts, which is significantly lower than what is expected from biologically processed phosphate. To date, most \( \text{P}_i \) found in soils and sediments in nature have been shown to be near or above their respective equilibrium values from PPase hydrolysis (Angert et al., 2012; Mizota et al., 1992; Tamburini et al., 2010), with \( \Delta \delta^{18}\text{O} \) (\( \text{PO}_4\text{-H}_2\text{O} \)), which ranges from 15\% to 25\% for most modern soils, depending on locations.

Thus, if a soil is developed from an igneous parent rock such as fresh basaltic flows or granite, the apatite-bearing igneous rocks will be physically and chemically broken down. If there were no biological activity, the soil, or more appropriately the regolith, would have \( \text{PO}_4^{3-} \) with the same \( \delta^{18}\text{O}_{\text{PO}_4} \) as that of the parent igneous rock. However, if
significant biological activity is operating in the weathering profile of an igneous rock, \( \text{PO}_4^{3-} \) will be cycled and the \( \delta^{18}\text{O}_{\text{PO}_4} \) will be equilibrated with ambient water, resulting in the increase of \( \delta^{18}\text{O}_{\text{PO}_4} \) of the bulk soil \( \text{PO}_4^{3-} \). It could be argued that some weathering profile may indeed be devoid of any life activity but accepts constant aeolian input of mineral dusts that contains \( \text{PO}_4^{3-} \) that may be biologically processed. In that case, an upward increase in \( \delta^{18}\text{O}_{\text{PO}_4} \) would also indicate somewhere on land there was an active land biota at the same time this lifeless weathering profile was developing.

1.4 Predictions and Testing Approach

Given that the oxygen composition of biologically equilibrated phosphate is independent of the original phosphate taken in (Blake et al., 2005), weathering profiles developing on igneous parent rocks would be best suited for this study. Therefore, I predict that the \( \delta^{18}\text{O}_{\text{PO}_4} \) will decrease downward through vertical weathering profiles approaching the igneous parent rock due to decreased amounts of weathering and reduced biological P-cycling of the igneous phosphate. To test this hypothesis I collected and analyze samples with different degrees of weathering across young weathering profiles that still retain their heavily weathered tops.

I selected two weathering profiles that have formed from a single igneous parent rock which are between 2-4 Ma. Samples are to be collected vertically across the profiles from sections with different degrees of weathering in order to demonstrate any changes in \( \delta^{18}\text{O}_{\text{PO}_4} \) due to an increase in bio-cycling. Sampling in high-vertical resolution has another purpose. It is uncertain if biological cycling of phosphate decreases monotonically with depth. Older weathering profiles are rarely preserved in geological records, let alone an entire weathering section from the very top to the fresh parent rock. Therefore, to use the
rare paleosols profiles preserved in old geological record, we need to examine recent soil profiles to determine how the $\delta^{18}O_{\text{PO4}}$ changes with depth, especially when it is approaching the parent rock.
2. MATERIALS AND METHODS

2.1 Weathering Profile Descriptions

The first of the weathering profiles studied, the Karamu basalt profile (Figure 2.1), is located in Karamu, approximately 17 km from Hamilton, New Zealand in an abandoned rock quarry. The Karamu basalt is part of the Okete Volcanics of the Alexandra Volcanic Group (Briggs and Goles, 1984) which has a reported age of 2.03 ± 0.03 Ma (Stipp, 1968). Weathering here extends to 66 m below the surface before reaching fresh basalt (Moon and Jayawardane, 2004). The profile subdivided into five sections, four weathered and one unweathered, according to visual observations in regards to the degree of weathering using the standards set forth by the New Zealand Geographical Society (Moon and Jayawardane, 2004). Samples collected by Jayawardane included bulk samples, core stone samples, and matrix samples from each section where applicable. The different sections of the profile were defined as follows: fresh rock—rock with no visible signs of weathering, slightly weathered—rock with some discoloration along defects, moderately weathered—rock that is discolored with some soil material present, highly weathered—containing more soil material than rock material but may still retain some core stones, and completely weathered: containing only soil material (Moon and Jayawardane, 2004; Society, 1988).

Petrographic analyses of samples by Moon and Jayawardane (2004) further revealed the mineralogical effects due to weathering; their analysis for each section of the profile are as follows. Fresh basalt samples as are fine-grained porphyritic rocks with euhedral to subhedral phenocrysts of olivine and titanaugite in a groundmass of plagioclase laths, glass and titanomagnetite. Other notable minerals included apatite (the primary source of inorganic P) and chromite. The only notable signs of weathering include
pale yellow discoloration on the crystal edges of some olivine crystals and some fine linear fractures in the groundmass.

Figure 2.1 – Map showing the location of Karamu, New Zealand and a sketch of the Karamu basalt weathering profile with depths for each of the weathering sections. Adapted from Moon and Jayawardane (2004).

Slightly weathered samples still retain the original porphyritic texture, however, clear signs of weathering have begun to appear. Olivine crystals have developed iddingsite
rims, a mixture of clays, iron oxides, and ferrihydrites. Titanaugite crystals have been fractured into smaller pieces and plagioclase crystals are beginning to take on a speckled appearance. Additionally there are increased amounts of fractures upon which glass from the matrix is beginning to weather into clays.

Moderately weathered samples contain the original porphyritic texture with some replacement. Olivine crystals have developed much larger iddingsite rims. Titanaugite crystals have begun to alter to hematite and most of the plagioclase has been converted to clays. Smectite is primary clay mineral with illite and kaolinite also present. Clays, goethite, and hematite fill much more evident cracks and fractures.

Highly weathered samples still contain fragments of the original rock and therefore, still contain some of the original porphyritic texture with some replacement. Titanaugite and Olivine have become completely replaced with only trace outlines of olivine crystals present. The rock primarily consists of clays, titanomagnatite, goethite and manganosite. Clay minerals include kaolinite, illite, halloysite, beidellite and montmorillonite.

Completely weathered samples consist mostly of clays with hematite and goethite also present. Traces of the original porphyritic texture and olivine still persist. Unfortunately, samples from this section did not yield sufficient PO_{4}^{3-} for analysis leaving only the three lower weathered sections to analyze.

The second weathering profile studied, the Hainan Island profile (Figure 2.2), is located in southern China in the northeastern part of Hainan Island in Wenchang County, about 60 km south of Haikou, the capital city of Hainan Province. It is exposed in a small hill freshly cut by a rock quarry (< 1 month) and has a maximum profile thickness of ~8 to 9 meters at the center of the hill (Ma et al., 2007). The profile is believed to represent
continuous weathering since deposition of the parent basalt at \(\sim 4\) Ma (Ma et al., 2007; Zhu and Wang, 1989). Samples analyzed for this study come from the lower most section of exposed profile extending from 3-4.5 meters down with the lower most sample located an estimated three meters above fresh basalt (Ma et al., 2007). Each of the samples comes from a section of the profile that is moderately to highly weathered with the lower three samples in a section that still contains core stones and the upper sample just above that in a section without core stones (Ma et al., 2007).

Figure 2.2 – Map showing location of Hainan Island, China along with a sketch of the Hainan basalt weathering profile showing depths and descriptions for each of the weathering sections. Adapted from Ma et al. (2007).
Petrographic analysis of the profile by Ma et al. (2007) show the parent rock to be a theolitic basalt consisting of 10% pyroxene phenocryst with a groundmass made up of 60% plagioclase, 25% clinopyroxene, and 5% ilmenite. Primary minerals are absent from the weathered sections of the profile with the exception of the core stones below 320 cm. They have been replaced with clays: kaolinite and halloysite, iron oxides: hematite and goethite, and aluminum hydroxide: gibbsite. The top 250 cm of the profile is a red laterite that fades to yellow between 250-320 cm from the top.

2.2 Phosphate Extraction

All samples were kindly provided by colleagues, Dr. Gang-Jian and Dr. Moon, for both weathering profiles were in a crushed powder form. Fresh basalt samples from the Karamu basalt profile were dark grey while all of the other samples were red to yellow orange, including the core stone samples. Bulk phosphate (e.g., mineral bound (apatite), oxide bound (Fe, Al), and dissolved phosphate) was extracted from approximately 5 mg of each sample powder with ~100 ml of 3M hydrochloric acid for three days (Figure 2.3). Similar methods of HCl extraction have shown that hydrolysis of organic PO$_4^{3-}$ in this step is negligible (Tamburini et al., 2010; Turner et al., 2005). Sample solutions were then transferred to 50 ml sample tubes and centrifuged for ten minutes to remove any residual solids. Whatman GF/F filters were used to vacuum filter the remaining solution followed by 0.2 μm syringe filter before being transferred into a beaker. They were then purified using methods adapted from Blake et al. (2010); McLaughlin et al. (2004); Tamburini et al. (2010) to remove organic matter before precipitation of silver phosphate.
2.3 Magnesium Induced Co-Precipitation

The first step in the purification process is Magnesium-Induced Co-precipitation (MAGIC) as was adapted from Karl and Tien (1992) to remove interfering ions, organic matter, and reduce the volume of the sample solution. Sample solutions were amended with 50-60 ml of 0.36M MgCl$_2$ •6H$_2$O and 3M NaOH was added until solution pH reached ~10 to induce the formation of brucite which is highly effective at scavenging PO$_4^{3-}$ from solutions (Karl and Tien, 1992). Solutions were then centrifuged to separate the brucite from solution after which it was dissolved with a minimum amount of 6M HNO$_3$. The solution was then diluted to 100 ml and followed by a second round of MAGIC and dissolution.

2.4 Ammonia Phosphomolybdate Precipitation

Next the sample solutions were precipitated as ammonia phosphomolybdate (APM) as adapted from Tudge (1960) and Tamburini et al. (2010) by first adding ~25 ml of 4.2M NH$_4$NO$_3$ and setting in a 48° C bath. This was followed by the gradual addition of 40 ml of ammonium molybdate solution (10 g of (NH$_4$)$_6$Mo$_7$O$_{24}$•4H$_2$O) 10 ml at a time. The solution was left in the warm bath for 2-4 hours before being removed and left to sit over night. The next day, APM crystals were collected via centrifuging and decanting the excess solution. The crystals were then rinsed 3-4 times with 0.6M NH$_4$NO$_3$ before being dissolved in a small amount (10-25 ml) of citric acid – NH$_4$OH solution (10 g citric acid, 140 ml of 30% NH$_4$OH, 300 ml distilled and deionized (DD) water) for the precipitation of magnesium ammonia phosphate.
2.5 Magnesium Ammonia Phosphate Precipitation

Magnesium Ammonia Phosphate (MAP) was precipitated according to methods adapted from Tamburini et al. (2010). First, 25 ml of magnesia solution (50 g MgCl₂•6H₂O and 100 g NH₄Cl are dissolved in 500 ml DD water, acidified to pH of 1 with 12M HCl, and then adjust the volume to 1:1 with DD water) was added. After which the solution should be alkaline and can be adjusted to a pH of 8 or 9 with 1:1 by volume NH₄OH/DD water. After letting the solutions sit over night the MAP crystals were extracted from the solution by centrifuging and decanting the excess solution. The crystals were then rinsed several times with 1:20 by volume NH₄OH/DD water to remove any chloride, which would produce insoluble AgCl during the precipitation of Ag₃PO₄ in the last step of the procedure. A small amount of HNO₃ (~5 ml) was then used to dissolve the MAP crystals in preparation for treatment with a cation exchange resin.

2.6 Cation Exchange Resin

Cation exchange resin was applied to remove interfering ions such as Mg²⁺, Fe³⁺. The resin was first rinsed 2-3 times with DD water and then conditioned with 7M HNO₃ on a shaker for 4 hours after which it was rinsed with DD water several times until the solution pH was neutral. Next ~5 ml of the resin was added for every 10 ml of sample solution and placed on a shaker over night to remove all cations from solution. The resin was then separated from solution and rinsed three times with DD water to ensure all phosphate had been removed from the resin. Solutions were then filtered through a 0.2 μm syringe filter and transferred to 50 ml centrifuge tubes.
2.7 Silver Phosphate Precipitation

Approximately 5 ml of Ag-amine solution consisting of 10.2 g AgNO₃, 9.6 g NH₄NO₃, 18.5 ml of concentrated NH₄OH and 81.5 ml of DD water (Tamburini et al., 2010) was added to the sample solution in preparation for the precipitation of silver phosphate (Firsching, 1961). Slowly HNO₃ droplets were added to bring the solution to a neutral pH where it formed a yellow precipitant of silver phosphate and left to sit overnight. Samples were then rinsed with DD water 3-4 times and dried in an oven overnight at 55°C.

2.8 Oxygen Isotope Analysis

Oxygen Isotope Analysis was performed at the Oxy-Anion Stable Isotope Consortium (OASIC) at Louisiana State University (LSU) on a Thermo Finnigan High Temperature Conversion Elemental Analyzer (TC/EA) set to 1450°C in continuous flow linked to a Thermo Finnigan MAT 253 Stable Isotope Ratio Mass Spectrometer. All samples were run in triplets when there was sufficient sample and all values are reported in VSMOW. An in-house standard OASIC-PO₄ was calibrated using two standards, UMCS-1 and AGPO-SCRI (Halas et al., 2011). For the calibration each of the three standards were ran one after the other in quadruplet and this was repeated for a total of three times. In addition NBS-120c was used to gauge the effectiveness of the extraction and purification procedure by testing for isotope fractionation caused by the extraction and purification procedure outlined above. It was found that measured values for NBS-120c were 0.8‰ heavier than the generally reported value of 21.7‰. Therefore a 0.8‰ correction was applied to all of the samples analyzed to correct for this discrepancy.
Figure 2.3 – Extraction and purification procedure used to obtain silver phosphate for analysis, from fresh and weathered basalts.
3. RESULTS

A total of nineteen samples were analyzed for the $\delta^{18}$O of phosphate, fifteen from the Karamu profile and four from the Hainan profile. Samples from the Karamu profile include five fresh basalt samples, four slightly weathered samples, three moderately weathered samples, and three heavily weathered samples. Each section of the profile included bulk samples plus representative samples of the rock material types found within the section: joint fill – weathered material filling joints between columns, core-stone – a weathering product formed from preferential weathering along joints and cracks, and matrix – highly weathered material surrounding core-stones. Samples from the Hainan profile include four bulk samples from the mid-section of the profile between 320 cm and 450 cm deep in an area that in moderately to highly weathered. The $\delta^{18}$O values for phosphate in the Karamu basalt profile samples range from 7.4-8.6‰ for fresh basalt, 19.4-22.2‰ for slightly weathered samples, 21-24.9‰ for moderately weathered samples, and 20-23.3‰ for heavily weathered samples (Figure 3.1). The Hainan island samples cover a similar range from 21.1‰ to 22.5‰ (Figure 3.2). A compiled table of the results for each profile can be found in Appendix A, Tables A.1 and A.2.
Figure 3.1 – Changes in $\delta^{18}$O$_{\text{PO4}}$ with depth for the Karamu, New Zealand weathering profile. Bulk values for each section along with matrix and core-stone values for the weathered sections are shown along side a sketch of the weathering profile. Sketch of profile adapted from Moon and Jayawardane (2004).
Figure 3.2 – Changes in $\delta^{18}O_{\text{PO}_4}$ with depth at Hainan, China, shown along side a sketch of the weathering profile. Sketch of profile adapted from Ma et al. (2007).
4. DISCUSSION

Data from this study are in agreement with previously reported low $\delta^{18}O_{PO4}$ values from igneous rocks $\sim$6-8‰ (Angert et al., 2012; Blake et al., 2001; Markel et al., 1994; Tamburini et al., 2010) and high $\delta^{18}O_{PO4}$ values from sediments and soils $\sim$15-24.8‰ (Angert et al., 2012; Mizota et al., 1992; Tamburini et al., 2010). There is also a partial agreement with the original hypothesis in the notable $\sim$3‰ difference in $\delta^{18}O_{PO4}$ between the matrix / joint fill samples and the corresponding core stone samples from each of the three weathered sections in the Karamu profile. This is likely because the core-stones have undergone less weathering than the surrounding material and may still contain some phosphate that has not been biologically processed and thus have a lower bulk $\delta^{18}O_{PO4}$. However, while the part of the original hypothesis that states the $\delta^{18}O_{PO4}$ of phosphate would increase moving up the profile was true, a gradual increase from fresh basalts to slightly weathered ones was not observed (Figure 3.1).

Instead of a gradual change in $\delta^{18}O_{PO4}$ up the Karamu profile, there is an abrupt 13.2‰ increase upon transitioning from fresh basalt to the slightly weathered section, within the lower 5 m of the 66 m profile. This indicates that phosphate scavenging by microbes is one of the first things to occur in the weathering process and is in agreement with observations that significant geochemical changes precede any observable mineralogical changes during the weathering process (Moon and Jayawardane, 2004; Price et al., 1991). In addition, data from the Hainan profile demonstrates a similar range of high $\delta^{18}O_{PO4}$ values, although the corresponding fresh basalt data are not obtained in this study. This confirms that significant microbial processing of phosphate occurs even in the lower most sections of the Karamu weathering profile where the rocks exhibit only minor
amounts of visible weathering. Therefore, even incomplete igneous weathering profiles should contain biologically cycled phosphate enriched in the $\delta^{18}\text{O}$ that contrasts against the low-$\delta^{18}\text{O}_{\text{PO}_4}$ igneous parent rocks, if microorganisms were present during their formation.

Although all of the $\delta^{18}\text{O}_{\text{PO}_4}$ values from this study were within the ranges previously reported for other basalts and soils, the isotopic composition of the weathered samples measured here are much higher than the equilibrium values expected through intracellular hydrolysis with PPase. For example, the estimated phosphate equilibrium values for the Karamu and Hainan profile are calculated to be $\sim 17\%$ and $\sim 15.5\%$ respectively. This was calculated using equation 2 above and plugging in the reported meteoric water values of $-5\%$ each and their average annual temperatures for their respective sample areas: $15^\circ\text{C}$ for Karamu and $24^\circ\text{C}$ for Hainan (IAEA, 2001). Based on these calculations each profile contains $\delta^{18}\text{O}_{\text{PO}_4}$ values up to $7\%$ over their expected equilibrium values. This indicates that intracellular equilibrium with ambient water in the soil may not be the dominant factor controlling the $\delta^{18}\text{O}$ of phosphate, but that there may be other enzymes or processes that exert significant influences.

Previous studies have also reported higher than expected $\delta^{18}\text{O}_{\text{PO}_4}$ values in soils (Angert et al., 2012; Mizota et al., 1992; Tamburini et al., 2010), however, they usually attempted to account for this using mixing of $\text{PO}_4^{3-}$ from other sources. This includes $\text{PO}_4^{3-}$ equilibrated with leaf water which is often highly enriched in $\delta^{18}\text{O}$ due to transevaporation (Angert et al., 2012), $\text{PO}_4^{3-}$ from animal bones, and $\text{PO}_4^{3-}$ equilibrated in ground water elevated in $^{18}\text{O}$ due to evaporation (Mizota et al., 1992). However, it is unlikely that these sources are applicable for our study materials given the depth at which these samples were collected. At 30-66 meters below the surface, significant leaching would be required to
bring PO$_4^{3-}$ equilibrated in leaf water or animal bone PO$_4^{3-}$ from the surface down to these depths and even then the fast rate of PO$_4^{3-}$ turnover by microbes (Paytan et al., 2002) should erase the previous δ$^{18}$O$_{PO4}$ signal. Evaporation has been shown not to have any affect at depth as enrichment in δ$^{18}$O of meteoric water in soils only occurs at <1m depth from the surface (Allison et al., 1983; Hsieh et al., 1998). Moreover, temperature should remain constant year round at depths below 10 m, roughly equal to the average annual temperature, so there would be no bias from higher biological activity during certain times of the year.

Sea salts may be a possible source of PO$_4^{3-}$ on land. Seawater contains ~0.088 ppm of PO$_4^{3-}$ which has an average δ$^{18}$O value of ~23‰ (Colman et al., 2005; Turekian, 1968). However, this is unlikely to be the case for our two weathering profiles for several reasons. First, airborne P-bearing particulates are dominantly from continental sources (Furutani et al., 2010; Graham and Duce, 1979) and therefore, any P-bearing sea-salts would be diluted by those from continental sources. Second, airborne marine P-bearing particulates are unlikely to be transported approximately 50 km inland to Karamu, New Zealand (Hossain and Easa, 2011). Last of all, if they did make it to Karamu the biological cycling of PO$_4^{3-}$ is fast relative to PO$_4^{3-}$ input by geological processes and the soil δ$^{18}$O$_{PO4}$ signal should be equilibrated with that ambient soil water (Paytan et al., 2002).

Another probable source contributing to the rather high δ$^{18}$O$_{PO4}$ in $P_i$ is a preferential uptake of $^{16}$O in PO$_4^{3-}$ by living organisms. Currently there is only one study demonstrating that this occurs, with a 3-4‰ fractionation observed during the uptake of P by E. coli (Blake et al., 2005). Therefore, at this time it is unknown what fractionation effects different microorganisms, plants, or fungi would have during P uptake. However,
given this was observed in one organism makes it likely that others will have similar behaviors, especially since living organisms are known for preferring lighter isotopes in other systems. However, to explain the high $\delta^{18}O_{PO_4}$ signals this way, we would have to expect a high fraction of the total soil $PO_4^{3-}$ to be bound to soil biomass, a situation not supported by the red and organic-poor soils we analyzed in this study.

One must also consider that there are many enzymes left to study for which their fractionation effects are currently unknown, such as the different classes of phytase enzymes. Phytase enzymes catalyze the hydrolysis of phytic acid and its salt, phytate (Mullaney et al., 2000), a major form of $P_O$ found in soils (Lim et al., 2007). Phytases are most commonly associated with fungi but they have also been found in plants and animals (Mullaney and Ullah, 2003) and recently, a new class of phytase enzymes, $\beta$-propeller phytase, was discovered in microorganisms (Lim et al., 2007). This enzyme is believed to play a major role in the breakdown of phytate and phytic acid in soils (Lim et al., 2007; Mullaney and Ullah, 2003) and therefore could have a significant influence on the $\delta^{18}O_{PO_4}$ in soils.

Furthermore, the fractionation effects in soils and weathering rocks may not be the same as what has been observed in laboratory studies. Therefore, at this time it is unclear what specific processes are elevating the $\delta^{18}O_{PO_4}$ above the expected equilibrium values. Nonetheless, all avenues point towards a biological origin and the range of values is distinctly different from that of $PO_4^{3-}$ in the parent igneous rocks, $\Delta\delta^{18}O$ (Parent-Weathered) $\sim 10\%$ on average. For the purpose of identifying biological activity during early Earth, we have established from the recent weathering profiles that the idea is viable.
5. CONCLUSIONS

Oxygen isotope analysis of phosphate in two young weathering basalt profiles reveals that significant biological processing of phosphate extends beyond the near surface soils all the way through the profile approaching the unaltered parent rock. It is apparent that only minor amounts of weathering can be accompanied by large changes in δ¹⁸O of phosphate in the presence of biological activities and that even incompletely preserved weathering profiles formed on igneous parent rocks can serve as a proxy for land biota in Earth’s past. Therefore, if microbial life on land does extend beyond the fossil record (Horodyski and Knauth, 1994) as indicated by other isotopic and geochemical biomarkers (Beraldi-Campesi, 2013; Gutzmer and Beukes, 1998; Martini, 1994; Neaman et al., 2005; Rasmussen et al., 2009; Stueken et al., 2012; Watanabe et al., 2000), then the δ¹⁸PO₄ profile of paleosols developed on igneous rocks should be an unambiguous indicator.
6. FUTURE WORK

I have tested two very recent soil profiles that are actively developing on basaltic parent rocks. However, working on paleosols has its own caveats. The most important issue is modern contamination either by recent microbial activity on outcrops or dust deposits. Therefore, to avoid data ambiguity, it is essential to use drill core materials for paleo-weathering profiles, because they are the best way to directly access fresh samples from buried paleosols. Therefore, it would be assuring if we examine one or two buried paleosols profiles before we move further back in time to test Precambrian soil profiles. It is also recommended that such a study include a higher sample resolution to create a better picture of how the δ¹⁸O changes across the transition from the parent rock to the slightly weathered section and steps should be taken to ensure that there is no post-sampling contamination either from biological activity or exposure to airborne P particles.

Table 1 — Potential locations to study recently buried igneous weathering profiles

<table>
<thead>
<tr>
<th>Geologic Setting</th>
<th>Location</th>
<th>Volcanic Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volcanic Islands</td>
<td>Hawaiian Islands</td>
<td>~1 Ma - Present</td>
</tr>
<tr>
<td></td>
<td>Galapagos</td>
<td>~4 Ma - Present</td>
</tr>
<tr>
<td>Rift</td>
<td>Taos, New Mexico</td>
<td>~2-4 Ma</td>
</tr>
<tr>
<td></td>
<td>Iceland</td>
<td>~15 Ma - Present</td>
</tr>
<tr>
<td></td>
<td>East Africa Rift</td>
<td>~20 Ma - Present</td>
</tr>
<tr>
<td>Large Igneous Province</td>
<td>Columbia River Basalt</td>
<td>~15 Ma</td>
</tr>
<tr>
<td></td>
<td>Deccan Traps</td>
<td>~65 Ma</td>
</tr>
</tbody>
</table>
Table 1 lists some locations of recently developed but currently buried soil profiles that developed on igneous rocks in different geological settings. Each of these locations contains weathering profiles that have been subsequently covered by basalts, a typical mechanism for burying and preserving basalt weathering profiles, adding another element to the study with an over capping igneous rock.

Once the buried yet relative young paleosols confirm my initial results, future work should focus on weathering profiles older than 500 Ma in order to provide new evidence of early microbial life on land. Some potential paleosols believed to be best suited for this investigation based on their age, parent rock, credibility as a continental deposit in origin, and previous evidence of biological activity are suggested here. They include the Elk Point paleosol (∼530 Ma) just outside Elk Point, South Dakota, the 1.1 Ga Keweenawan Rift paleosols in upper Michigan and Minnesota, and the Transvaal Supergroup paleosols in South, Africa: the Schagen paleosol (∼2.7 Ga), and the Heckpoort paleosol (∼2.25 Ga).

The youngest of the group, the ∼530 Ma Elk Point paleosol, would be a good place to begin. It is from a time just before the evasion of land by plants and there is already fossil evidence to suggest that microbial life on land had been established by this time. In addition, this paleosol has already demonstrated evidence of biological activity via the loss of phosphate near the top of the weathering profile (Horodyskyj, 2009; Horodyskyj et al., 2012). Therefore it is strongly suspected that this weathering profile will contain biologically cycled PO$_4^{3-}$ with a much higher δ$^{18}$O$_{PO_4}$ than the metagabbro on which it has developed. If so it would further validate this as a viable method and provide more evidence of life on land in the Early Cambrian. In addition this paleosol is located deep
beneath the surface and there are already drill core samples available at the South Dakota Geological Survey core repository.

Following in age is 1.1 Ga Keweenawan Rift paleosols in the Lake Superior region of Michigan and Minnesota. They include the Sturgeon Falls paleosol, the Good Harbor-Bay paleosols, and the Centennial Mine paleosol. Each of these paleosols has developed on basalts or sediments derived directly from basalts that erupted during the rifting event. The Sturgeon Falls paleosol located near Strugeon Falls, Michigan developed on a basalt from the Siemens Creek formation in an arid environment (Mitchell and Sheldon, 2010; Zbinden et al., 1988). However, this paleosol is only known from outcrop at one location and it is remote and hard to access. Therefore obtaining drill core may prove difficult. The Good Harbor-Bay paleosols are found in a road-cut that near Good Harbor-Bay, Minnesota and is easy to access yet they are a series of thin paleosols that include lacustrine environments that is not strictly terrestrial (Mitchell and Sheldon, 2009). Last is the Centennial Mine paleosol. It is located in near Houghton, Michigan and is found within the Houghton and Hecla conglomerate, one of the many interbedded conglomerates of the Portage Lake Volcanic series. This paleosol is believed to have formed on an alluvial plane composed of igneous sourced sedimentary rocks in an arid environment (Kalliokoski and Welch, 1985) and would be the best paleosol from this time period for this study as it is located deep below the surface safe from modern contamination, and therefore drill core would need to be obtained.

Moving further back in time, the Transvaal Supergroup of South Africa contains some of the oldest known terrestrial paleosols that have developed on a variety of igneous rocks (Martini, 1994). Two of these profiles look very promising and have already
demonstrated evidence of early life on land (Watanabe et al., 2000; Neaman et al., 2005) making it likely that there would be biologically cycled PO$_4^{3-}$ in these profiles as well. The first profile, the ~2.7 Ga Schagen paleosol developed on a dunite and is up to ~17 m thick (Watanabe et al., 2000). This profile contains organic carbon that is believed to be from a microbial mat that formed on the soil surface in a semi arid environment (Watanabe et al., 2000). The second profile is the ~2.25 Ga Heckpoort paleosol that developed on the Heckpoort basalt which is up to ~18 m thick and located over 100 m below the surface in some areas (Yang and Holland, 2003). It is also believed to have formed in a dry climate and contains signs of biological activity based on element mobility (Fe, P, Cu, Al) within the profile (Neaman et al., 2005). In addition drill core already exist for these profiles making it an easy place to conduct this study. Finding biologically cycled PO$_4^{3-}$ from either of these profiles would provide more evidence to strengthen the case that microbial life on land began very early in Earth’s history.
REFERENCES


Stipp, J.J., 1968. The geochronology and petrogenesis of the cenozoic volcanics of North Island, New Zealand, Australian National University, Canberra.


APPENDIX: δ\textsuperscript{18}O\textsubscript{PO4} DATA

Table A.1 - Karamu basalt weathering profile δ\textsuperscript{18}O\textsubscript{PO4} analysis

<table>
<thead>
<tr>
<th>Weathering</th>
<th>Sample Type</th>
<th>Sample</th>
<th>δ\textsuperscript{18}O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly Weathered 30m-46m</td>
<td>Core-stone</td>
<td>MPJ-50</td>
<td>20.0‰</td>
</tr>
<tr>
<td></td>
<td>Bulk</td>
<td>MPJ-47</td>
<td>20.1‰</td>
</tr>
<tr>
<td></td>
<td>Matrix</td>
<td>MPJ-41</td>
<td>23.3‰</td>
</tr>
<tr>
<td>Moderately Weathered 46m-56m</td>
<td>Bulk</td>
<td>MPJ-17</td>
<td>23.5‰</td>
</tr>
<tr>
<td></td>
<td>Core-stone</td>
<td>MPJ-16</td>
<td>21.0‰</td>
</tr>
<tr>
<td></td>
<td>Matrix</td>
<td>MPJ-15</td>
<td>24.9‰</td>
</tr>
<tr>
<td>Slightly Weathered 56m-66m</td>
<td>Joint fill</td>
<td>MPJ-52</td>
<td>22.2‰</td>
</tr>
<tr>
<td></td>
<td>Bulk</td>
<td>MPJ-51</td>
<td>22.0‰</td>
</tr>
<tr>
<td></td>
<td>Bulk</td>
<td>MPJ-14</td>
<td>21.7‰</td>
</tr>
<tr>
<td></td>
<td>Core-stone</td>
<td>MPJ-8</td>
<td>19.4‰</td>
</tr>
<tr>
<td>Fresh Basalt &gt;66m</td>
<td>Bulk</td>
<td>MPJ-7</td>
<td>8.6‰</td>
</tr>
<tr>
<td></td>
<td>Bulk</td>
<td>MPJ-6</td>
<td>7.4‰</td>
</tr>
<tr>
<td></td>
<td>Bulk</td>
<td>MPJ-4</td>
<td>7.6‰</td>
</tr>
<tr>
<td></td>
<td>Bulk</td>
<td>MPJ-3</td>
<td>8.5‰</td>
</tr>
<tr>
<td></td>
<td>Bulk</td>
<td>MPJ-2</td>
<td>8.5‰</td>
</tr>
</tbody>
</table>

Table A.2 - Hainan Island weathering profile δ\textsuperscript{18}O\textsubscript{PO4} analysis

<table>
<thead>
<tr>
<th>Weathering</th>
<th>Sample Depth</th>
<th>Sample</th>
<th>δ\textsuperscript{18}O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly Weathered 320cm</td>
<td>HK06-10</td>
<td>21.7‰</td>
<td></td>
</tr>
<tr>
<td>Moderately Weathered 400cm</td>
<td>HK06-15</td>
<td>21.1‰</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HK06-17</td>
<td>22.5‰</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HK06-20</td>
<td>21.5‰</td>
<td></td>
</tr>
</tbody>
</table>
VITA

Dustin Warn Boyd is from Searcy, Louisiana, and graduated from Jena High School in 2002. He then attended Northwestern State University in Natchitoches, LA, where he graduated Magna Cum Laude with an Associate of Science in Electronics Technology and a Bachelor of Science in Industrial Engineering Technology in May of 2006. After graduation he began work for Schlumberger as a Wireline Engineer in Rock Springs, WY. During his course of employment with Schlumberger he spent four months training in Amreya, Egypt, and one month in Edinburgh, Scotland. In 2009, Dustin resigned from Schlumberger to return to school to study Geology. He chose Louisiana State University because he believed it was the best place for both him and his wife, Shirene, to further their education as she would go on to graduate with a Masters of Art in Elementary Education in 2013. Upon his acceptance to LSU Dustin initially studied undergraduate geology before transitioning to the graduate program in 2012. During his time at LSU he was able attend the Geology Field Camp in Colorado Springs, Colorado, the Geology in Iceland course, and do field work in New Mexico, Upper Michigan, and Minnesota. Upon graduating from LSU, Dustin plans to move to Midland, TX, and work as a Geologist for Concho.