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Influence of Common Bean (Phaseolus vulgaris) Grown in Elevated CO2 on Apatite Dissolution

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INFLUENCE OF COMMON BEAN (PHASEOLUS VULGARIS) GROWN IN ELEVATED CO_2 ON APATITE DISSOLUTION

By

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An Abstract of the Thesis Presented
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Elevated concentrations of atmospheric CO_2 brought about by human activity creates changes in plant morphology, growth rate and exudate production. Our study sought to understand the effect of these changes on soil mineral weathering using plants grown under two conditions, ambient CO_2 (400ppm) and elevated CO_2 (1000ppm). *Phaseolus vulgaris* (common beans) were grown in flow-through microcosms consisting of a mixture of quartz and apatite sands. Plant growth was sustained by a nutrient solution devoid of calcium (Ca) and phosphorous (P). Using Atomic Adsorption Spectroscopy and colorimetry, Ca and P content of the leachate and plant tissue served as a proxy for apatite dissolution. Plants were harvested periodically during the 8-week experiment to show Ca and P content with time. *P. vulgaris* grown in elevated CO_2 had a greater root to shoot ratio. This outcome was expected based on the results of many other studies. The planted microcosms were found to have a lower pH than abiotic controls 811% more Ca was released from biotic than abiotic experiments by the end of week 8. The presence of plants resulted in the release of over 100× more P compared to their absence. Plants

grown in elevated CO_2 released 82% more Ca and 80% more P than those grown in ambient conditions. Although elevated CO_2 helped plants to grow larger root structures and lower the solution pH, no significant change to weathering rates was observed during the experiment. Our results show the importance of below ground carbon fluxes in creating changes to the rhizosphere which aid in P release from apatite

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1. INTRODUCTION

The rate at which minerals weather to release ions and form soils is a complex process mediated by both abiotic and biotic sources of weathering. Mineral weathering releases ions which are an important source of plant nutrients. Release of cations such as magnesium (Mg) and calcium (Ca) also serve as a carbon dioxide (CO_2) sink through the formation of carbonate minerals. Recently an increasing amount of attention has been paid to the role of plants increasing the rate of soil mineral weathering (Akter & Akagi, 2005b; Andrews et al., 2008; Calvaruso et al., 2013).

Plant metabolic processes are sustained by the collection of nutrients through physical and chemical alteration of substrates. Although plants receive the bulk of their alimentation from soils, they require carbon (C) as a source of new tissue material. Through photosynthesis, CO_2 is used as a building block for different plant structures. Increases in atmospheric CO_2 levels lead to enhanced growth and below ground C allocation in the majority of land plant species (Li et al., 2003; Luo et al., 2006; Yan, et al., 2006). In a world where human activity continues to increase the concentration of atmospheric CO_2, it is important to quantify potential changes in plant mediated weathering of minerals.

We hypothesize that elevated atmospheric CO_2 will lead to both increased root growth and organic acid exudation. These two traits will lead to improved acquisition of phosphorus (P) derived from apatite. In order to test this hypothesis, we will:

1) Determine the dissolution rate for apatite in the presence of *Phaseolus vulgaris* 'Langstrath Stringless' (green bean) grown at 400 ppm and 1000 ppm CO_2.

2) Determine the dissolution rate for apatite weathered abiotically at 400 ppm and 1000 ppm CO_2.

3) Describe physical and chemical characteristics of precipitates and changes to surface morphology throughout the experiment.

1.1 Effects of Elevated CO_2 on Plant Physiology and Growth

The changes plants undergo in atmospheres containing elevated CO_2 have been extensively studied (Drake et al. 1997; Li et al., 2003; Luo et al., 2006; Yan et al., 2006). Plants use carbon from the air in order to construct new tissue. Increases in atmospheric concentration of CO_2 leads to more photosynthesis in some plants. Autotrophs take CO_2 from the air and reduce it to form organic carbon. This reduced carbon is used to create new body tissue as well as energy storing sugars. In C3 carbon fixation, plants rely on the enzyme ribulose bisphosphate carboxylase (RuBisCO) to facilitate the conversion of CO_2 to organic C. C3 plants represent 80% of earth's gross productivity, and are common in both agricultural and natural ecosystems (Wand et al., 1999).

At a pCO_2 of 400 ppm, C fixation enzymes are under saturated. This inefficiency in the presence of existing concentrations of CO_2 allows for C3 plant growth to be enhanced by elevated CO_2. In the construction of new plant tissue RuBisCO and CO_2 from the air are combined to produce a 3-carbon compound called phosphoglyceric acid (PGA). Due

to the abundance of oxygen in the atmosphere, 25% of RuBisCO is oxygenated rather than carbonated (Busch, 2013; Sharkey, 1988). The oxygenation of RuBisCO is known as photorespiration. Photorespiration occurs after the plant has already consumed two moles ATP and NADPH. The reaction consumes energy and results in the production of no organic C. Using four different methods of measuring photorespiration, Sharkey (1988) observed photorespiration to drop to 12% in an atmosphere containing 800 ppm CO_2. It has been found that these losses to photorespiration are no longer measurable in bean plants when the concentration of CO_2 reached 1200 ppm. Further increases in CO_2 showed no significant increase in growth (Jolliffe & Ehret, 1985).

In elevated CO_2 atmospheres, photorespiration decreases and C3 plants become more efficient. A reduction in waste products created by photorespiration lowers the plant's N demands as less N containing enzymes are needed to process oxygenated RuBisCO. Plants require less water due to the closing of stomata. The closing of stomatal openings in leaves leads to lower rates of transpiration and higher soil moisture (Drake et al., 1997). Increased soil moisture has been shown to intensify physical weathering and sediment transport (Gedney et al., 2006; Raymond & Cole, 2003). The increase in physical weathering creates more surface area and thus encourages chemical weathering (Chen et al., 2014; Gaillardet et al., 1999).

Some studies show benefits from elevated CO_2 are temporary in systems that are nitrogen (N) and P limited (Lloyd & Farquhar, 1996; Norton, et al., 1999). Thus, it is possible that nutrient availability largely controls how an ecosystem responds to elevated

CO_2. In other instances N and P limitations are mitigated by a greater efficiency in their use (Conroy, 1992; Lloyd & Farquhar, 1996). In a survey of 156 different plants grown in elevated CO_2, Poorter (1993) found the largest increase in growth rate came from N-fixing C3 plants and those that formed a mutual relationship with N-fixing organisms. Plants that were assisted in nutrient acquisition were able to assimilate a larger quantity of C than plants limited by nutrient demands. In some instances, plants respond to elevated CO_2 by increasing symbiotic association with fungi and bacteria (Soussana & Hartwig, 1995). Haase et al. (2007) found a 177% increase in exudation of malate in bean plants grown in elevated CO_2. Malate serves as a chemo-attractant designed to attract N-fixing microbes. In a review of 60 different experiments Drake et al. (1997) found photosynthesis to increase by 58% in doubled CO_2 concentration when compared to plants grown in ambient conditions.

Chemo-attractants are just one example of carbon-based organic acids which increase in elevated CO_2. C-based exudates are also used to aid in nutrient acquisition and protect plants from heavy metal toxicity. These organic acids are derived from energy producing reactions known as, “dark reactions” or, “light independent reactions”. In areas of the plant which are unable to produce energy from photosynthesis, such as the roots, sugars are oxidized to produce energy through glycolysis. During the breakdown of sugars, different enzymes used produce oxalate, malate and citrate. Increases in exudate production under elevated CO_2 and nutrient stresses have been observed (Phillips et al., 2009, Delucia, 1997, Haase et al., 2007, and Johansson et al., 2009). In a study by Fransson and Johansson (2010), C assimilation was found to increase by 41-47%

between plants grown in ambient (400 ppm CO_2) and elevated conditions (800 ppm CO_2). The increase in C assimilation was complimented by a 120-160% increase in organic acid production.

During glycolysis, oxidation of sugars also releases CO_2. This results in soil CO_2 concentrations being several orders of magnitude higher than those in the air (Oh & Richter, 2004). In natural ecosystems respiration outside of the plant is a large source of soil CO_2. Soil microbiota are responsible for decomposition of organic matter (OM) as an energy source. Infiltration from ambient pCO_2 is small compared to these subsurface factors. Under elevated CO_2, production of soil CO_2 increases due to a greater input of OM which fuels microbial populations (Parton et al., 1995). In a study by Andrews and Schlesinger (2001) elevated CO_2 resulted in a 27% increase in soil respiration. Changes to microbial communities and fungi living within the rhizosphere can be strongly controlled by plant root exudation. Studies on changes in rhizosphere populations have failed to consistently show an increase or decrease in microbial populations under elevated CO_2 (Lipson et al., 2005).

1.2 Inorganic Nutrients

All requisite nutrients for plant growth, other than C and N, initially come from the weathering of minerals. Macronutrients such as K, Ca, Mg, P, S, and micronutrients Cl, Fe, B, Mn, Zn, Cu, Mo and Ni are common components of minerals in earth's crust. Plants absorb these elements for different metabolic processes and temporarily immobilize them in organic forms. In order to attain nutrients, plants have developed a

variety of tools to chemically alter the area surrounding the roots, the rhizosphere, and liberate different ions. Recent studies have shown that land plants facilitate nutrient release from minerals (Calvaruso et al. 2013, Hinsinger et al. 2001, Marschner & Römheld 1983). Plants have been shown to increase weathering by 1.5-10× over abiotic controls (Calvaruso et al., 2013; Hinsinger et al., 2001; Uroz, et al., 2009).

Mineral weathering can be accelerated by the release of exudates from plant roots. Production of organic acids, sugars, amino acids and enzymes allows plants to change the soil chemistry in their immediate surroundings. These changes affect the redox conditions, microbial populations, surrounding biomass and chemical speciation of elements (Katoh et al., 2015). Akter and Akagi (2005) used fine mesh bags to prevent roots from coming into contact with mineral grains, showing that even at a distance of several millimeters, chemical changes within the rhizosphere are effective in altering mineral surfaces.

1.3 Biotic Mechanisms Involved in Mineral Dissolution

Mineral dissolution in soils is strongly controlled by soil pH (Mengel and Kirkby, 1987). As early as 1902, scientists have observed etching on calcite grains caused by roots (Deherain, 1902). The presence of roots can drive the pH of the rhizosphere to as low as 3 (Berner et al. 2004).

Acidification of the rhizosphere occurs through three separate mechanisms: proton flux due to cation exchange; generation of carbonic acid through root respiration; and exudation of organic acids (Hinsinger, 1998; Zhu et al., 2014). Proton flux is caused by

the imbalance of anions and cations being taken up by plants. If excess cations are taken up, the root compensates by releasing the surplus positive charge as protons. Oppositely, if more anions are taken up by the root, plants will secrete OH^- to maintain an internal charge balance (Haynes, 1990; Marschner & Römheld, 1983; Nye, 1981). Because N is consumed in greater quantities than other nutrients, the consumption of NH_4^+ and NO_3^- largely causes these internal imbalances (Haynes, 1990). The generated OH^- and H^+ are released into the rhizosphere via fine roots (Marschner & Römheld, 1983). At low pH, H^+ adsorbs to the surface of minerals and facilitates exchange of Na, K, and Ca. Mineral dissolution also occurs when the pH is high, resulting in the formation of metal-hydroxides (Chaïrat et al., 2007). When the pH is in the neutral range, both H^+- and OH^--mediated dissolution mechanisms become less effective at weathering minerals. This neutral pH range is common in natural environments other than the root zone.

Uptake of ions by plants reduces their solution saturation and creates an imbalance according to Le Chatelier's principle (Law of Mass Action). This depletion of the soil solution leads to further mineral dissolution. In experiments by Houben & Sonnet (2012) plant uptake of Zn cations from solution resulted in further dissolution of smithsonite.

1.4 Organic Acids and Mineral Dissolution

In the neutral pH range, H^+- and OH^--mediated processes have a lower influence on mineral dissolution. The effect of organic acids becomes more pronounced as it can also promote mineral dissolution through the formation of surface complexes. Although organic acids help lower the pH, they also accelerate mineral weathering by forming

covalent bonds through the sharing of electrons. The most commonly studied organic acids are polyfunctional aliphanitic anions. Organic acids such as citrate, malate and oxalate owe their effectiveness in weathering to their structure which has multiple functional groups. This allows for the formation of soluble multidentate complexes. The formation of surface complexes weakens and breaks the bonds between metals and oxygen atoms (Van Hees et al., 2002; Xiao & Wu, 2014; Zhu et al., 2014). Ligands can also accelerate weathering through the formation of complexes with cations in solution, which reduces their saturation state of the fluid (Uroz et al., 2009). Conversely, metal-ligand complexes can form precipitants on mineral surfaces and inhibit dissolution (Welch & Vandevivere, 1994).

The effectiveness with which organic acids can break down minerals is dependent on their concentration and subsequent adsorption to mineral surfaces. The adsorption of ligands is dependent on metal speciation and charge of the mineral surface (Ullman et al., 1996). The rate of mineral dissolution can be increased by surface dislocations and impurity defect sites where reactive sites are more accessible (Dorozhkin, 1997). Additionally, high concentrations of cations and anions can compete with organic acids and mineral surface reaction sites (Biber & Stumm, 1994).

Dissolved organic carbon (DOC) is present in the soil at concentrations of 2-30 ppm, but can reach higher levels within the rhizosphere (Drever, 1994). Grierson (1992) found concentrations of DOC in the rhizosphere soil solution of *Banksia integrifolia* as high as 2500 ppm. This concentration was an order of magnitude higher than that in the

surrounding soil solution. The majority of DOC consisted of citric and malic acid. Oxalate, malate and citrate can be found in forest soil solutions ranging in concentrations between nM to μM (Ullman & Welch, 2000). Plant species modify their rhizospheres to different degrees. Katoh et al. (2015) investigated Pb mobility in the presence of two plant species from the same ecosystem. It was found that buckwheat, a Pb tolerant species was able to alter the speciation of Pb contained in the mineral, pyromorphite, through the exudation of oxalate. This caused the release of Pb into solution. Hairy vetch, a Pb intolerant species had no effect on lead mobility and showed symptoms of Pb toxicity. Several studies (Drever, 2005; Martínez-Alcalá et al., 2010; Shen et al., 2002) also hypothesize that organic acid production can be increased as a response to phytotoxic elements.

Organic acids show selectivity for specific cations. This can be seen in the formation of etching pits on the surface of minerals. In an experiment evaluating the effect of four different crop species (lupine, oilseed rape, maize and banana) on basalt dissolution, Hinsinger et al. (2001) found that Ca and Na were released one to five times faster than other elements, indicating minerals were weathered preferentially.

1.5 Phosphorus in Natural and Fertilized Ecosystems

Although most soils around the world contain abundant P, the quantity that is bioavailable often limits plant growth (Martin, 1979). Soil P is contained in three pools: organic (P_o), inorganic (P_i) and dissolved in soil solution. P_o is contained in organic detritus or immobilized by microbes (Table 1-1). P_o is the largest pool of soil P and can

represent up to 80% of total P. Even though P_o cannot be directly absorbed by plant roots, fungi and plants are known to produce enzymes to facilitate its release (Gyaneshwar et al., 2002). The second largest pool of soil P is inorganic (P_i) and is found adsorbed to minerals surfaces, precipitated on soil particles, or imprisoned by the crystal structure of minerals. P_i cannot be used by plants until it is solubilized through changes to rhizosphere chemistry. The smallest P pool is dissolved orthophosphate contained in the soil solution. These dissolved nutrients move towards the roots due to concentration gradients created by plant uptake (Gauch, 1972).

Table 1-1. Concentrations of P in different soil pools at 0-10 cm.

Pool	Approximate P concentration
Soil Solution	1-3 μM m^{-3}
Organic P	100-400 kg ha^{-1}
Inorganic P	50-200 kg ha^{-1}

This depletion of dissolved P allows for more P to be released from labile sources, such as desorption from charged surfaces, dissolution of minerals and hydrolysis of P_o. Jungk (1991) observed this process happening at a rate of 0.13 μM per day. Because of this slow release, up to 20% of total costs associated with farming can be due to P fertilizer.

1.6 Apatite Weathering

In non-fertilized ecosystems plants attain P from mineral sources. A large mineral pool of P is in apatite ($Ca_5(PO_4)_3$ (OH,F,Cl)), which can be found in igneous, metamorphic and sedimentary rocks. The dissolution of apatite also releases less common elements such as Zn, Mo, B and Cu that are needed for many plant processes (Filippelli, 2008).

Apatite dissolution kinetics have been well studied due to its importance as a P source in nature (Chien et al., 1980; Chien, 1977; Christoffersen et al., 1978; Guidry & Mackenzie, 2003; Valsami-Jones et al., 1998). As the most common phosphate mineral, apatite is the foundation of the P cycle (Filippelli, 2008). All organisms have a P-based energy currency and need it to develop new tissue (Rakovan & Pasteris, 2015). It is present in DNA, RNA, ATP and ADP (Welch, 2002). The term apatite represents more than 40 distinct minerals. Most commonly found in nature, fluorapatite (FA) and hydroxyapatite (HA), are calcium phosphates containing F and OH respectively (Back, 2015).

The dissolution of apatite is controlled by solution saturation, hydrodynamics and pH. The structure of HA and FA consists of orthophosphate tetrahedra linked by two different Ca polyhedra. The first Ca site consists of four Ca atoms in six-fold coordination and the second site consists of six Ca atoms in seven-fold coordination (Figure 1). The F^- or OH^- occupies a space in the second Ca polyhedra (Dorozhkin, 2012).

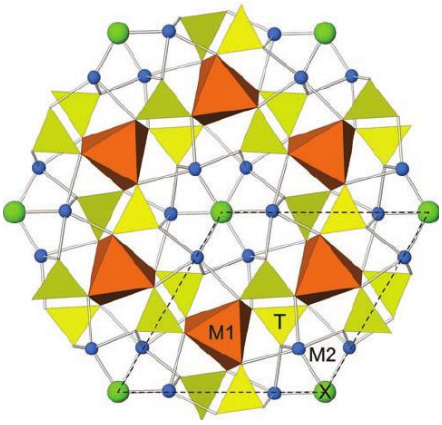


Figure 1-1. Crystal structure of apatite. The dotted lines outline one-unit cell which consists of M1-centred Ca polyhedra, represented as six-fold coordinated metaprisms, M2 Ca (two of the seven bonds overlap in this projection), yellow phosphate tetrahedra and the dominant anion shown in green (Pasero et al., 2010)

Based on the crystal structure, the release of P is dependent on the destruction of bonds between Ca-O and Ca-F^-/OH^-, whereas destruction of P-O bonds is not required for apatite dissolution.

Harouiya et al. (2007) preformed a series of batch reactor experiments to evaluate dissolution rates of apatite in temperatures ranging from 5 to 50 °C and pH ranging from 1 to 6. The released concentrations of Ca, P and F were stoichiometric. Stoichiometric apatite dissolution results in a Ca/P ratio of 1.67 (Christoffersen et al., 1978). The rate equation for apatite dissolution calculated using data from multiple studies is $R=10^{-6.64}*a_H^{-0.541}$ (Brantley et al., 2008).

Organic acids have been shown to accelerate apatite dissolution through the same mechanisms described above (Calvaruso et al., 2013; Welch et al., 2002; Margolis & Moreno, 1992). Phosphorus release is related to an organic acid's ability to form

complexes with a particular metal, in this case Ca. Solubility constants serve to predict the likelihood of complexation of metals in the presence of different ligands.

Welch et al. (2002) conducted a series of batch reactor studies using organic acids and microbes to evaluate the influence of microbes on apatite dissolution. It was found that acetate and oxalate accelerate mineral dissolution by an order of magnitude. Organic acids lowered the pH and formed complexes with Ca in solution and on the mineral surface. In the same study, microbes produced oxalate and accelerated weathering by two orders of magnitude while maintaining at near neutral pH.

Studies by Christoffersen et al. (1996 and1998) model the dissolution of apatite by the formation of weathering pits, which are caused by the removal of a Ca, F or PO_4. These studies show the spreading of weathering pits is controlled by the saturation state of Ca in solution.

Uroz et al. (2009) conducted a field-based study of apatite dissolution using mesh bags placed in the rhizospheres of different beech trees. The forest was chosen for its naturally Ca and P poor soils, which allowed for easy observation of nutrient fluxes from fluorapatite. This plot was compared to a similar forest that had been fertilized with Ca and P. Dissolution was measured by change in sample weight. After four years, samples had lost between .4% and 1.8% of their weight in unfertilized plots. Observation of individual grains under an SEM revealed three times as many weathering pits on the surface of mineral grains retrieved from the unfertilized than those from the fertilized plots.

1.7 Phaseolus vulgaris

The legume *Phaseolus vulgaris* 'Langstrath Stringless' (green bean) produces a broad spectrum of organic acids. It goes through an entire growth cycle and fruits in 6-8 weeks (Helm, 1990). Growth cycles consist of four different stages and are characterized by development of different plant structures and nutrient demands. During the first stage, establishment, limited plant-soil interaction occurs, as the plant is not yet involved in photosynthesis. The following two stages require nutrients to be attained from the plant's substrate. After establishment, plants enter the vegetative stage, which is discernable by the development of cotyledons. During this time plants have a high nutrient demand and will be producing more biomass above ground than below. The plants will then undergo a reproductive stage where growth occurs mostly below ground. *P. vulgaris* is a member of the *Fabaceae* family and known to be an autogam, meaning that it is able to fruit without the help of pollinators (Cobert et al., 2011).

In a survey of exudates produced by plant roots of different species, Vančura and Hanzlíková (1972) found that *P. vulgaris* produced a broad spectrum of organic acids at a large quantity per unit weight. Oxalic, citric, malic, lactic and succinic acids were found in the rhizosphere of *P. vulgaris.* Legumes are known to acidify their rhizosphere through the preferential uptake of NH_4 over NO_3 (Caixan, 2003). In an evaluation of four different bean varieties of bean plants, Shen et al. (2002) found that they respond to P deficiency through the production of citrate, tartrate and acetate. The same study found that bean plants, which produced more organic acids, were able to accumulate higher concentrations of P in plant tissues.

P. vulgaris was successfully used in nutrient cycling studies by (Cobert et al., 2011; Guo et al., 2005). As a C3 plant, *P. vulgaris* will respond to changes in pCO_2 with changes in photosynthetic efficiency. A study by Cowling and Sage (1998) found a 77% decrease in biomass of beans grown at 200 ppm CO_2 compared to 380 ppm CO_2. Rao (2015) also tested changes to plant physiology under elevated CO_2 using *P. vulgaris*. It was found bean plants grown in 580 ppm CO_2 had a higher dry weight, produced more flowers and a greater root mass than plants grown at 380 ppm.

2. MATERIALS AND METHODS

2.1 Materials

The legume *P. vulgaris* was chosen for its small size and short lifecycle. *P. vulgaris* plants have also been used in elevated CO_2 studies by Haase et al. (2007), Cobert et al. (2011) and Rao et al., (2015). Seeds purchased from Everwilde Farms® were washed in a 2 m H_2O_2 solution for 20 min and rinsed with deionized water to remove microbial contaminates (Akter & Akagi, 2005; Van Tichelen et al., 2001). Once cleaned, seeds were germinated on sterile cotton mats. After emergence of taproot, plants were transferred to 60 mL syringes which served as plant pots.

2.1.1 Minerals

Apatite crystals purchased from Madagascar Minerals in Tucson, Arizona, were crushed using a jaw crusher and ground to sand using a disk pulverizer. This preparation equipment is located in the basement of Bryand Hall. Prepared sands were dry-sieved to obtain a size fraction between 0.5 and 1mm. This size fraction was chosen to match experiments used to define kinetic constants of minerals in field ecosystems (Augusto et al., 2000; Marie-Pierre et al., 2009). The apatite sand was combined with quartz sand (1.2-2 mm) donated by US Silica. Close evaluation of the provided sand shows the presence of feldspar grains (~20-30%). The quartz and apatite mixture was similar to the growing medium used by Calvaruso et al. (2013) and Andrews et al. (2008). This mixture created a substrate that would retain sufficient moisture as well as have enough mineral

surface area to measure mineral dissolution. A mixture of 85% quartz with 15% apatite by weight was used to fill plant pots. The quartz grains served as an inert root anchoring material and the apatite was the only P and Ca source for the plants. Particle surface area of apatite was too large to be measured using the surface area analyzer at the Laboratory for Surface Science and Technology (LASST). Instead, grains surfaces were analyzed and determined to be free of surface pores. Geometric surface area was then calculated under the assumption grains were spherical, using the equation:

$$Surface\ Area = \frac{V_s * \rho}{SA} \qquad \text{Equation 1}$$

where V_s is the volume of a sphere, ρ is the density of apatite and SA is the surface area of a sphere. Because grains were sorted using sieves, apatite particles used in the experiment ranged from 0.5-1mm. Using the averaging method from Tester et al. (1994), a weighted average particle diameter can be calculated assuming a flat particle size distribution (Equation 2):

$$D_e = \frac{D_{max} - D_{min}}{\ln\left(\frac{D_{max}}{D_{min}}\right)} \qquad \text{Equation 2}$$

where D_e is the effective diameter used in surface area calculations, and D_{max} and D_{min} are the upper and lower limits of the particle size range. Apatite grains were calculated as having a surface area of 26.1 $cm^2\ g^{-1}$(Equation 1). Quartz and apatite grains used in the experiment were cleaned using ethanol and a Misonix S-4000 Sonicator the procedure of Adcock et al. (2013). Quartz grains were first hand rinsed twice in 100 mL of ethanol and

then ultrasonicated for 10 minutes replacing the ethanol every two minutes. The process continued until the supernatant was clear. Apatite grains were sonicated for 2 min at amplitude eight for four minutes and at amplitude six for 1 min to remove fine particles. Before filling the plant pots, the sand was autoclaved for 20 min at 120 °C, three days before starting the experiment. Calvaruso et al. (2013) also used an autoclave to sterilize their quartz-apatite mixture and found no change to dissolution kinetics.

A sample of crushed, sieved and sonicated apatite was sent to ACT Labs in Ontario, Canada, to quantify concentrations of major elements using X-ray fluorescence (XRF). This combined with observations of mineral grains using a scanning electron microscope were used to confirm the apatite used in the experiment were homogeneous and free of inclusions.

2.1.2 Nutrient Solutions

In order to sustain healthy plant growth, minimal nutrients were added to the constant drip solution (Table 2-1). Similar solutions were used to sustain plants in studies by Akter & Akagi (2005), Hinsinger et al. (2001) and Uroz et al. (2009). Both cation and anion sources of N were added to avoid inducing H^+ fluxes and artificially influencing solution pH. Calcium and P were not added to the nutrient solution to encourage plants to use mineral sources for these nutrients.

Table 2-1. Nutrient solution used in experiment.

Salt	**Ion**	**Desired Concentration (mg L^{-1})**	**Salt added (mgL^{-1})**	**mM**
KCl	K	235.00	448.09	6.011
$MgSO_4 + 7H_2O$	SO_4	189.72		1.975
	Mg	48.00	486.77	1.975
H_3BO_3	B	0.0001	0.00005	$8.5X10^{-7}$
$FeSO_4$ + 7H2O	Fe	0.0010	0.0050	$1.8X10^{-5}$
$MnCl_2$+4H2O	Mn	0.50	1.80	0.009
	Cl	211.26		6.029
NH_4NO_3	NO_3	464.77		7.496
	NH_4	135.23		7.496

2.2 Experimental Design

2.2.1 Environmental Growth Chambers

Six 20-gallon fish tanks with the dimensions of 24” W × 12” D × 16” H were fitted with air-tight lids by the University of Maine Advanced Manufacturing Center to create the environmental growth chambers (EGCs) where the concentration of CO_2 could be held at approximately1000 ± 200 ppm (Figure 2-1). Photos of growth chambers can be found in the Appendix section I.

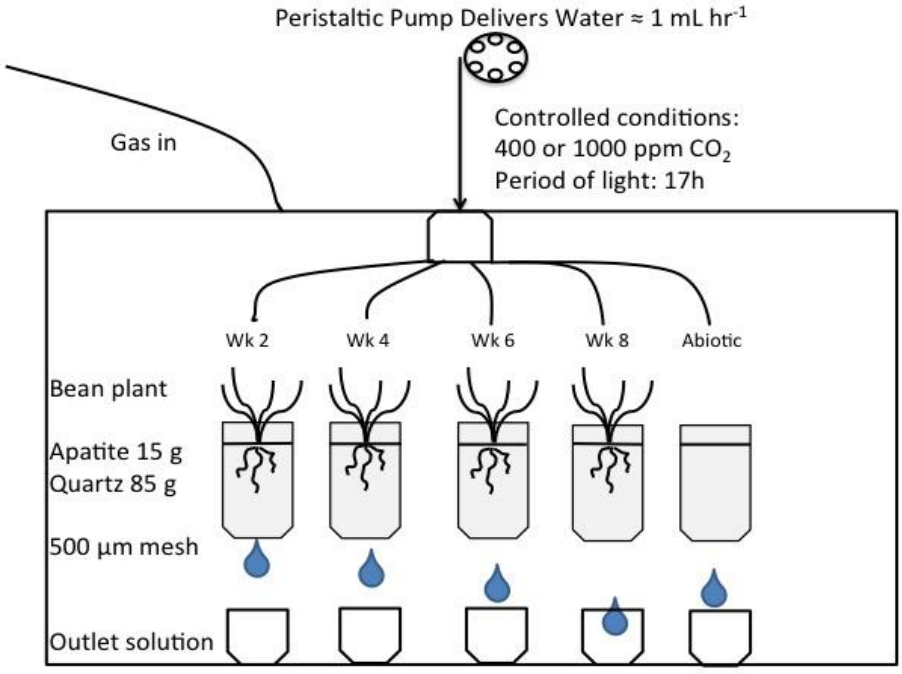


Figure 2-1. Schematic of environmental growth chambers built by the University of Maine Advanced Manufacturing Center.

The CO_2 concentration was elevated using Hydrofarm Desktop CO_2 Monitors (Autopilot) which were modified to control a solenoid valve. The firmware in the CO_2

monitor allows for programmable alarms consisting of LEDs indicating if the concentration of CO_2 is lower than, equal to, or higher than a desired level. By exchanging the "low concentration" LED with a bipolar junction transistor, the circuit responsible for supplying power to open a solenoid valve (Figure 2-2) can be completed and the valve opens. When the solenoid valve opens, concentrated CO_2 flows into the EGC until the pCO_2 of the environment reaches 1000 ppm and the voltage supplied to the transistor is shut off and the valve closes.

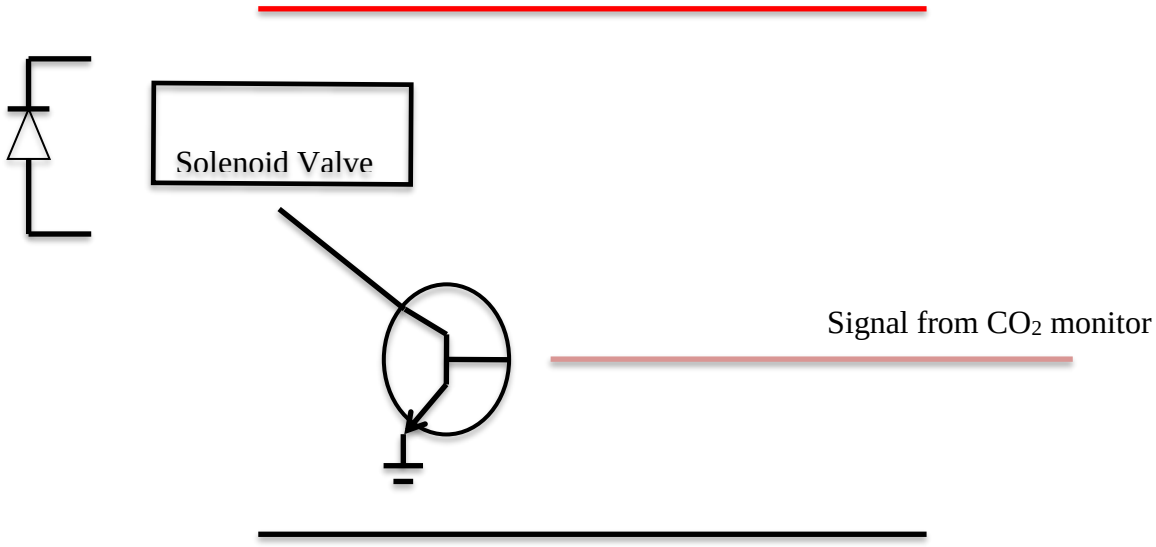


Figure 2-2. Wiring diagram of modifications made to CO_2 monitor.

In order to avoid hysteresis created by slow diffusion of CO_2 in the EGC and the time needed for the CO_2 monitor to take measurements, CO_2 was added to each EGC at a flow rate of 0.4 $ft^3\ hr^{-1}$ using a rotameter manufactured by Omega Instruments. Even after these precautions are taken, there was still a ± 200 ppm range observed while conducting

trial experiments. By using 3-way solenoid valves one tank of compressed CO_2 could be used on multiple EGCs.

Each CO_2 condition (400 and 1000 ± 200 ppm) was replicated in three EGCs. Replicates contained four plants as well as an abiotic control that contained the soil media but no any plants. One plant was harvested and analyzed for Ca and P content every two weeks (2, 4, 6 and 8 weeks) in order to capture the kinetics of plant growth and nutrient uptake.

2.2.2 Flow Through Microcosms

Plants were grown in flow though microcosms similar to those described by Calvaruso et al. (2013). Using peristaltic pumps to create constant flow of liquid ensured that the concentration of ions in solution remains constant and below solution saturation throughout the experiment. Plants were grown in 60 mL syringes filled with 15 mg of apatite and 85 mg of quartz sand. The plants were supplied with a nutrient solution dripping at a constant rate of approximately 1 mL hr^{-1} (~2.5" per week) due to the high permeability of sand using an ultra-low flow peristaltic pump (The Control Company). This rate was based on the beans' need to receive between .5" and 2.5" of water per week to sustain healthy growth (Houdek & Stradler, 2016).

A piece of 500 μm nylon mesh was placed at the base of each syringe to allow solution to escape while preventing the loss of mineral grains. The leachate was collected in beakers and weighed to estimate its volume. Each week approximately 30 mL of solution was collected for quantification of total P and Ca.

2.2.3 Sampling

Leachate samples were collected weekly during the experiment (Table 2-2). On each sampling date ~30 mL of solution was collected from all microcosms to determine pH, flow rate, and concentrations of Ca and P. Samples were collected using a 0.45 μm filter and acidified with 0.3 mL 15.8 M HNO_3 and stored at 1 °C (Greenberg, 2005). Plant tissue was collected every two weeks by harvesting one plant from each environment and digesting it to measure uptake of ions by the plants. By sampling the system periodically, release of ions with respect to time was measured. Once the plants were harvested, collection of leachate was discontinued. Because apatite was the only source of Ca and P, the sums of Ca and P found in the leachate and plant tissue can be used to calculate a dissolution rate for apatite (Calvaruso et al., 2013). The data presented in the results are shown as a moving average of three simultaneously conducted replicates. They can be categorized as one of the four following categories: biotic experiments receiving elevated CO_2 (B_e), abiotic experiments receiving elevated CO_2 (Ab_e), biotic experiments receiving ambient CO_2 (B_a), and abiotic experiments receiving ambient CO_2 (Ab_a). Until week 4, all treatments were conducted in triplicate. By week 6, two plants had died and one replicate of the ambient conditions was discontinued in order to replace plants which had died.

Table 2-2. Sampling totals for 8-week experiment.

Sample type	Replicates	Sample dates	Total
Seed Ca	1	1	1
Seed P	1	1	1
Plant Ca	6	4	24
Plant P	6	4	24
Leachate Ca	30	8	164
Leachate P	30	8	164

2.2.4 Release Rates

The weathering budget was defined as the amounts of Ca and P collected by the plant and released into solution (Calvaruso et al., 2013),

$$W = L + I \qquad \text{Equation 3}$$

where W is the total P or Ca released from apatite (mg sec^{-1}), I is the amount of ions immobilized by the plant (mg sec^{-1}) at sampling time t (sec) and L is the concentration in the outlet solution with time (calculated in Eq. 4), assuming a constant flow rate:

$$L = Q \times C \qquad \text{Equation 4}$$

L has the units of mg sec^{-1}, where C is the concentration (mg L^{-1}) and Q is the leachate discharge rate measured in L sec^{-1}. The rate of mineral dissolution was calculated using the rate formula proposed by Calvaruso et al. (2013).

$$R=\frac{W}{SA\times M\times\theta\times V_p\times t} \quad \text{Equation 5}$$

where R is the rate (mol m^{-2} s^{-1}), W is the weathering budget based on P or Ca concentrations, SA is the surface area of the mineral grain (cm^2 g^{-1}), M is the mass of apatite (g), θ is the pore space saturation, t is time elapsed in (s) and V_p is a stoichiometric coefficient based on the ratio of Ca to P.

Due to evapotranspiration taking place in biotic experiments, θ was calculated individually for each harvested pot. First, the unsaturated bulk density was calculated using the weight of sand added to the plant pot divided by the volume. Because the plant pots were syringes, the volume could be read directly off the side of the pot. An average porosity was approximated using the density of the crushed rock divided by dry bulk density.

$$\Phi=1-\frac{\rho_{Crushed\ Rock}}{\rho_{Bulk\ Rock}} \quad \text{Equation 6}$$

where Φ represents average porosity. Because the plant pots were filled with a mixture of 85% quartz and 15% apatite by weight, the densities used reflect the quantities of the two materials. With a known unsaturated porosity, any decrease in porosity is caused by pore space being filled with water or plant roots (Equation 7). Once the shoots were removed, the plant pots were reweighed to an approximate total pore space filled by

leachate and plant roots. The mass of plant root mass was estimated using the assumption that dry weight of roots is 7% of fresh weight (Ryan et al., 2001).

$$\theta = m_{total} - m_{sand} - m_{root} - m_{pot} \quad \text{Equation 7}$$

2.3 Analytical Methods

2.3.1 Plant Tissue Oxidation and Digestion

Root and shoot tissues were processed separately because the concentrations of Ca and P is differs between plant structures (Hewitt & Smith, 1974). This is the standard in most plant tissue data sets and allows for comparison between studies. Plant tissue was dried at 70 °C for 2 days to determine the dry weight (Thomas, 2013). The dry weights were used to normalize elemental concentrations per unit weight. Once dried, plants were ground up using the Wiley Mill in the MAFES lab in Deering Hall at the University of Maine to homogenize the sample. Plant tissue was ashed using a muffle furnace at 550 °C for 6 hours. After cooling, the plant tissue was wetted with DI water and solubilized using 5 mL of 5.83 M HCl and boiled on a hotplate for 30 min. After cooling, plant samples were strained using a 0.45 µm filter and diluted up to 50 mL (Chapman & Pratt, 1961). This processing ensures that all forms of P are oxidized and hydrolyzed to form orthophosphate. Before starting the experiment, ten seeds were digested together and analyzed using the same process described above in order to characterize initial concentrations of elements in seeds.

2.3.2 Quantification of Ca Using Atomic Absorption Spectroscopy

Calcium content of leachate and digested plant tissue was measured using atomic absorption spectroscopy (AAS) on a Thermo S-Series AAS in the CES Environmental Engineering Lab at the University of Maine. Total Ca was measured using light absorbance at 422.7 nm in samples ionized in an acetylene flame. A calibration curve was constructed on each sampling date using a stock solution made from 0.2497 g $CaCO_3$ powder which was dried at 180 °C for 1 hr. After drying the $CaCO_3$ was dissolved in 10 mL of concentrated HCl and then diluted up to 1000 mL of DI water. Standards ranging in 0-60 mg L^{-1} were prepared and resulted in a linear relationship between concentration and absorbance. For every 20 samples a replicate and an external quality check were analyzed to ensure that data was reproducible and meaningful. This procedure follows the methods outlined by Eaton et al. (1998).

2.3.3 Colorimetric Determination of Total P

The P content of digested plant tissues and liquids was evaluated using the Varian Cary 50-Spectrophotometer in Sawyer Hall's Water Chemistry Lab. This spectrophotometer can accommodate a 10 cm cell allowing a detection range from 1 to 200 ppb for P. Most samples had concentrations of 10 to 2000 ppb and were diluted to 10% of their original concentration. The measurement requires that all forms of P present be digested in an acidic environment to release it as orthophosphate (H_3PO_4). In order to ensure that all P was in the quantifiable form, orthophosphate, leachate was acidified using 1 mL of 11 N H_2SO_4 and oxidized using 1 mL of 1.75 M ammonium persulfate.

The treated samples were then placed in an autoclave at 15 psi and heated to 121 °C for 30 minutes (Eaton et al., 1998). By adding ammonium molybdate, molybdophosphoric acid is formed and reduced using ascorbic acid to generate a blue color (Murphy & Riley, 1962). The reduced P complex absorbs light at 880 nm.

A calibration curve was constructed on each sampling date using a stock solution made from 0.2197 g KH_2PO_4 powder which was dried at 180 °C. After drying, the salt was added to 1000 mL of milliQ water to make a 1000-ppm stock solution. Standards ranging from 1 to 100 μg L^{-1} were prepared at each sampling date and resulted in a linear relationship between concentration and absorbance. For every 20 Samples an older sample was retested and a quality check made from an external P standard was measured to ensure reproducibility and assure the quality of the calibration curve.

2.3.4 Sample Corrections

When concentrations of Ca and P were higher than the upper limit of the calibration curve, samples were diluted and the concentration was multiplied by the reciprocal of the dilution factor (Equation 8).

$$C_c = C_d \times D_f \qquad \text{Equation 8}$$

where C_c stands for corrected concentration, C_d stands for the diluted concentration and D_F represents the dilution factor. The D_F accounts for the aliquot of sample taken from the total volume and the volume of reagent added. In the case of total P all samples were diluted to 7.5 percent of their original concentration to remain within the linear range of the calibration curve, and to account for the volume of colorimetric reagents added.

2.3.5 Mineral Content of Shoots

To check the nutritional status of plants and estimate concentrations, samples of plant tissue were analyzed using the inductively coupled plasma atomic emission spectrometer (ICP-AES) in Deering Hall at the University of Maine. This also served as a second method for measuring Ca and P and provided an external quality check.

Comparing concentrations of P from ICP analysis to colorimetry show higher values for P were recorded colorimetry. Shoot tissue samples had 6318 and 6444 ppm according to colorimetry, where values reported by ICP were 5420 and 5830 ppm. Calcium concentrations measured by AAS were much lower than values reported by ICP. The two samples measured by ICP had concentrations of 1640 and 1600 ppm Ca where values recorded by AAS had 802 and 461 ppm Ca. One possible reason for is due to the amount of dilution plant samples underwent during digestion. The absorbance values for Ca in the shoot tissue fell just above the minimum detectable levels.

2.3.6 Batch Reactor Dissolution

Two experiments were conducted to assess the stoichiometry of abiotic apatite dissolution in the absence of quartz grains. Five grams of cleaned apatite grains were added to 50 mL of nutrient solution and shaken in a bath for 2 weeks at 25 °C. Concentrations of Ca and P were measured from 10 mL samples for week 1 and 2.

2.3.7 Post-Experiment Mineral Experiments

Mineral grains were collected from the biotic pots at the time of harvest. Grains of apatite and quartz from abiotic experiments were sampled after the 8-week experiment had ended. Samples were examined with scanning electron microscopy (SEM) and data were collected using electron dispersive spectrometry (EDS) and backscatter electron (BSE) imagery. Fresh samples were also examined for comparison. Before examination, samples were air-dried and carbon-coated. Observations of quartz and apatite surfaces were made using the Tescan Vega XMU Scanning Electron Microprobe located in Bryand Hall. The use of the SEM allowed for imaging of changes to surface topography, and the possible formation of secondary precipitates. Using the EDAX Apollo EDS detector, chemical analysis of spots on mineral grains and semi-quantitative chemistry data were collected to help identify secondary mineralogy.

2.3.8 Statistics

Experiments were run in triplicate and averaged to help reduce the inherent variability present in biological experiments. In order to compare the averages, data were evaluated

using Student's T-test. If variance was shown not to differ significantly ($P>0.05$) using an f-test, the variance for the elevated and ambient conditions could be pooled. The arithmetic means for data were compared using a two tail unpaired t-test. A calculated t-statistic could be compared to critical values for t found in Portney & Watkins (2000). When a calculated t-statistic didn't show a difference in means ($P>0.1$) a p value for the null hypothesis was also reported.

3. RESULTS

3.1 XRF Analysis of Apatite

X-ray fluorescence analysis (XRF) of apatite grains used in this study shows that concentration of Ca is higher than that found in typical apatites. This elevated Ca concentration creates a Ca to P ratio of 1.80 (Table 4). Apatite used in experiments by Calvaruso et al. (2013) and Park et al. (2005) had a lower Ca concentration and a Ca to P ratio of 1.67.

Table 3-1. Composition of apatite used in experiments. Moles cation refers to stoichiometry of one mole of apatite.

	Weight %	Moles Cation
Ca	41.465%	5.2176
P	17.753%	2.8905
Si	3.022%	0.5426
F	3.455%	0.9172
Na	0.065%	0.0002
Fe	0.061%	0.0055
Mn	0.033%	0.0030
V	0.002%	0.0002
Ti	0.002%	0.0002

3.2 Changes in pH with Time

The pH values used in data analysis were the average of three replicates in each treatment (Figure 4). Weekly pH values can be found in Appendix A. The pH of the nutrient solution was 5.01 after mixing and equilibrating with the air. The pH of all outlet solutions increased until week 4. After week 4 the average pH of B_e pots fell while the three other treatments, B_a Ab_e Ab_a remained high. Using a t-test it was determined that

there was no significant difference between the average pH of leachate sampled from B_e and B_a pots until week 5 ($p<0.05$ appendix H-7). At week 5, leachate from B_e had an average pH of 4.57±0.16, which was significantly lower than all other conditions. For the final two weeks of the experiment the average pH of B_e was significantly lower ($p=0.008$ and $p=0.03$ respectively) than the pH measured in the B_a experiments.

The average pH of Ab_e and Ab_a experiments did not differ significantly at any point during the experiment. The average pH throughout the 8-week experiment of Ab_e and Ab_a experiments were 5.18 ±0.07 and 5.07 ± 0.08 respectively.

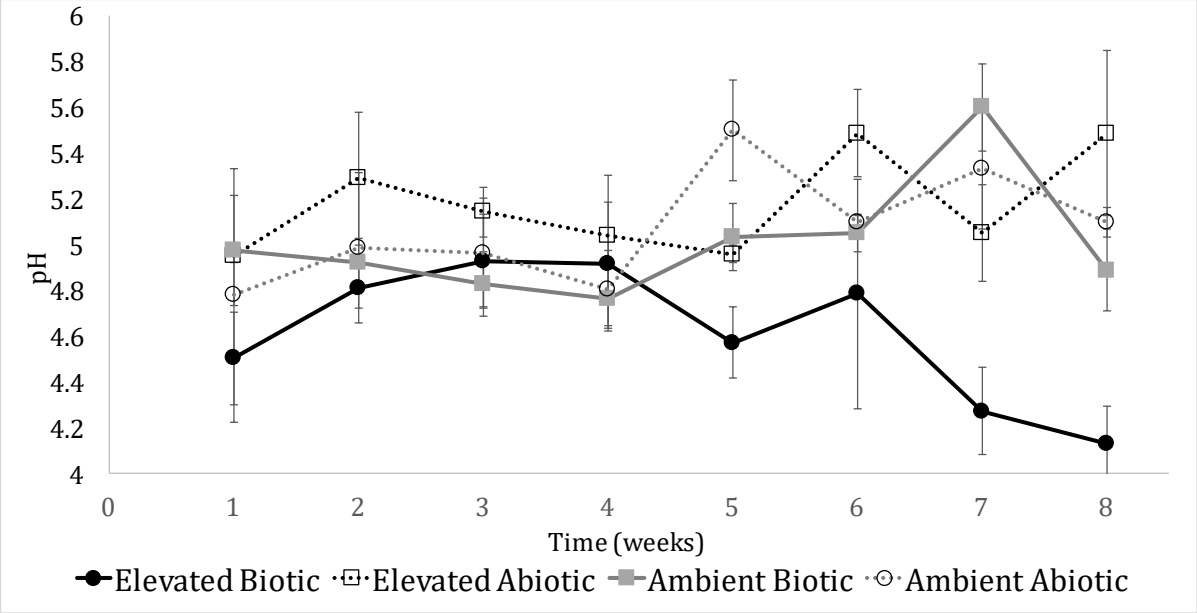


Figure 3-1. Average pH of the outlet solutions. Error bars represent the standard deviation of the mean.

3.3 Plant Growth

Dry weights of plant roots and shoots were measured after harvesting (Appendix B). Although the average total dry weight of plants grown in elevated CO_2 was greater than the average weight of ambient plants at each sampling point, the difference was not statistically significant during any of the 4 weeks ($p>0.1$) (Table 3-2). A t-test showed plants grown in elevated CO_2 had a significantly higher root to shoot ratio throughout the experiment. Analysis of the root to shoot ratio is a common way to normalize root growth for comparison among non-identical plants (Munns et al., 2010) ($p<0.05$, Figure 3-2). Statistics comparing average plant weights can be found in appendix H-3.

Table 3-2. Dry weights of total plant tissue recorded in grams.

	Dry weight (g)	**Average wt. (g)**	**±**		**Dry weight (g)**	**Average wt. (g)**	**±**
Week 2	0.4387	0.3809	0.041	**Week 2**	0.3463	0.3203	0.015
Elevated	0.2820			Ambient	0.3299		
	0.4220				0.2847		
Week 4	0.2420	0.3201	0.035	**Week 4**	0.2915	0.4768	0.114
Elevated	0.3872			Ambient	0.7492		
	0.3312				0.3896		
Week 6	0.2599	0.6082	0.298	**Week 6**	1.1271	0.7760	0.248
Elevated	1.3388			Ambient	0.4249		
	0.2259						
Week 8	1.1716	2.2366	0.753	**Week 8**	1.3279	1.4503	0.087
Elevated	3.3015			Ambient	1.5726		
	0.3077						

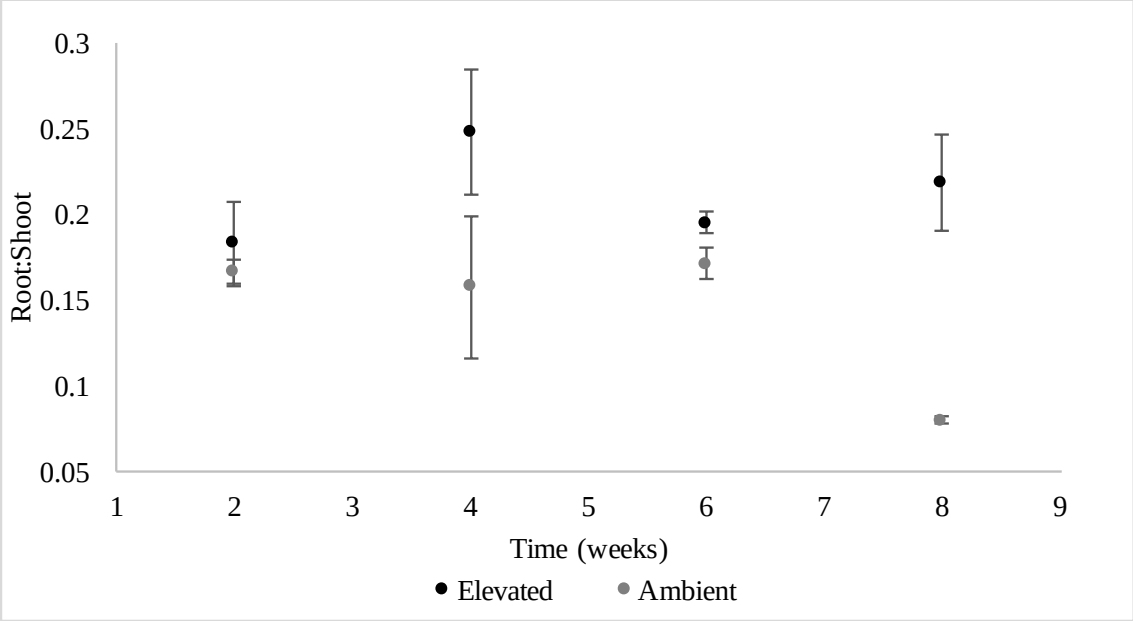


Figure 3-2. Ratio of roots to shoots throughout 8-week experiment. Error bars represent the standard deviation of the mean.

3.4 Plant Nutritional Status

Nutrient content of the plant shoots was analyzed to evaluate the possibility of nutrient deficiencies (Table 3-3). By comparing concentrations of macro- and micro-nutrients to average concentrations collected for *P. vulgaris* by Benton et al. (1996), nutritional status of plants was quantified for one plant grown under ambient and elevated CO_2 treatments at week 8. It was found that both sets of plants were deficient in Ca, Al and Zn at Week 8.

Table 3-3. Concentrations of mineral elements (ppm dry matter) in elevated and ambient plant shoots. Healthy range comes from Benton et al. (1996).

	Elevated (ppm)	Ambient (ppm)	Healthy Range (ppm)
%N	5.11	6.40	3-6
Ca	0.16	0.16	0.80-3.0
K	3.85	4.98	1.8-4.0
Mg	0.42	0.64	0.3-1.0
P	0.54	0.58	0.3-.0.8
Al	36.50	44.20	624.00
B	22.20	36.90	20-75
Cu	6.95	7.45	5-30
Fe	83.50	109.00	20-200
Mn	89.90	105.00	30-300
Zn	8.08	10.20	20-200

3.5 Distribution of Ca pools

Calcium released from apatite in biotic experiments was either incorporated into plant tissue or dissolved in the leachate (Equation 3). The majority of Ca was contained in leachate, reaching as high as 92% On average, elevated plant tissue retained a higher percentage of Ca than tissues of plants grown in ambient CO_2 (Figure 3-3). A larger percentage of Ca was lost to the effluent in both elevated and ambient conditions later in the experiments.

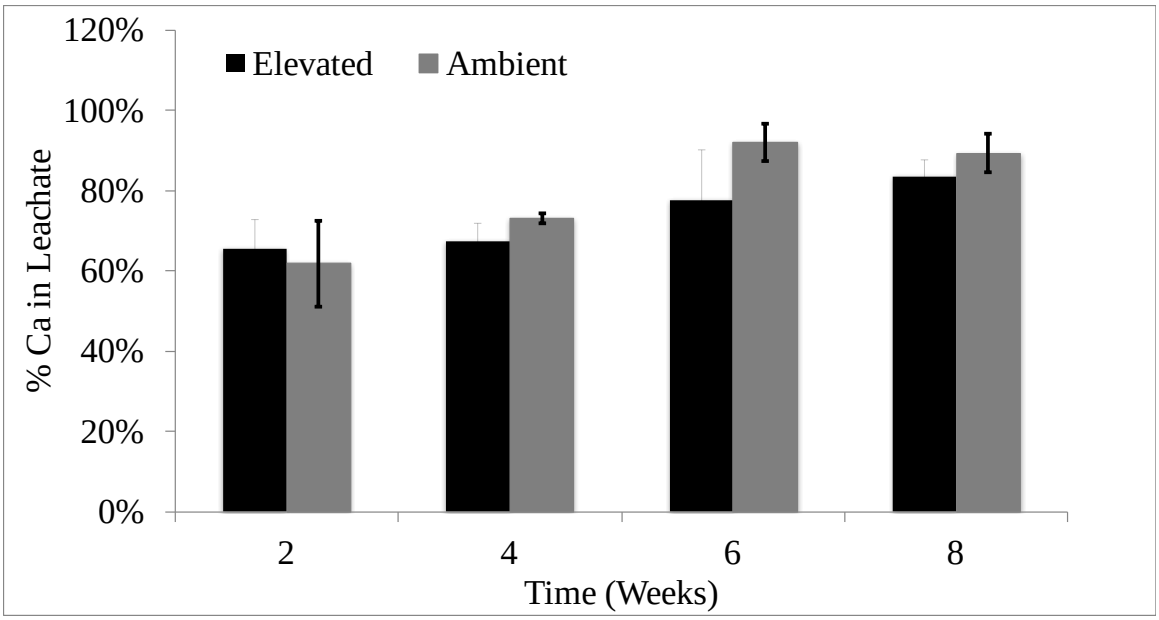


Figure 3-3. Percent total Ca found in leachate.

In biotic experiments, dissolved Ca concentrations varied by week and ranged between 20-140 µmoles of Ca (Figure 3-4) Comparing the averages of total dissolved Ca, a t-test shows that significantly more Ca was released from Be experiments at weeks 6, 7 and 8. During other weeks, no significant difference was observed between the two biotic conditions. By the end of week 8, Be experiments had released 82% more Ca to the leachate than Ba experiments (Table 3-4). Statistics comparing the average Ca content between experiments can be found in appendix H.

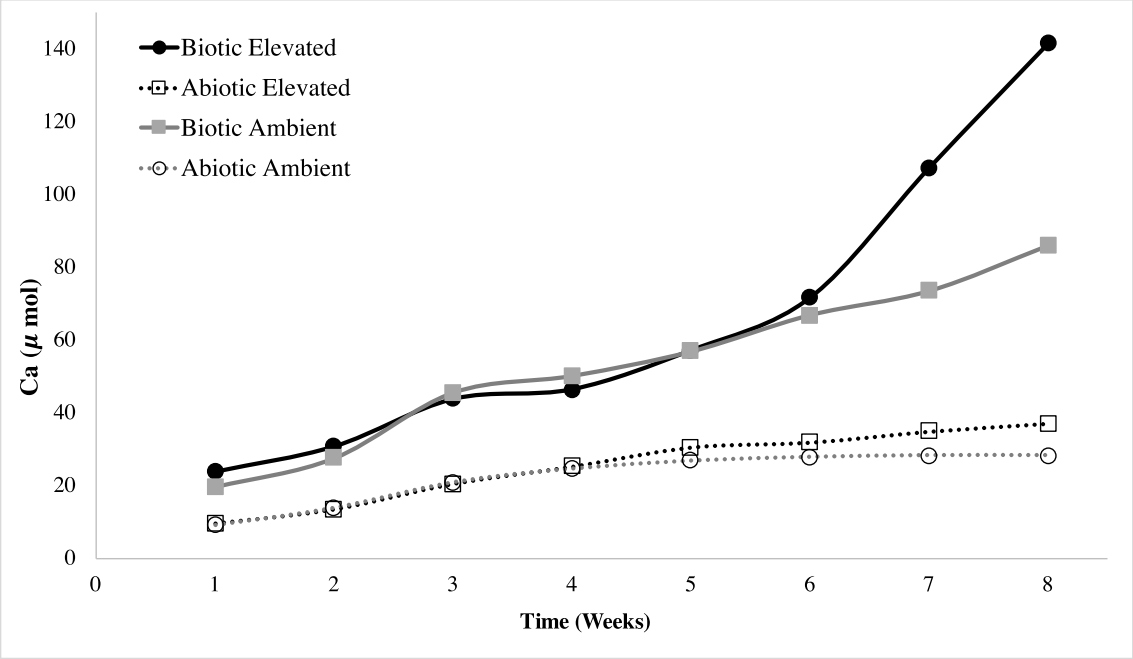


Figure 3-4. Moving average of total Ca (μmol) measured in the outlet solution during the 8-week experiment.

Table 3-4. Ca concentrations (µmoles) measured in leachate during 8-week experiment.

Chamber No.	Column	Week 1 Ca (µmol)	Week 2 Ca (µmol)	Week 3 Ca (µmol)	Week 4 Ca (µmol)	Week 5 Ca (µmol)	Week 6 Ca (µmol)	Week 7 Ca (µmol)	Week 8 Ca (µmol)
Elevated 1	1*	8.40	5.87	7.17	5.44	4.78	1.87	1.46	1.28
	2	16.79	9.50	18.49	13.33	11.59	16.27	35.65	39.18
	3	14.78	10.99	18.64	8.03	4.15	1.65		
	5	18.94	5.60	12.17	8.31				
	6	18.25	9.50						
Elevated 2	7*	10.23	3.40	6.54	3.85	3.67	0.53	4.41	3.30
	8	16.56	5.62	20.28	9.61	24.33	24.51	0.00	0.00
	9	17.58	8.77	27.59	15.81	27.20	37.19	65.94	53.90
	11	11.63	10.36						
	12	14.37	9.44	6.97	7.38				
Elevated 3	13*	9.58	2.49	7.51	4.85	7.54	2.15	3.22	2.02
	14	32.34	3.73						
	15	77.70	4.05	4.73	3.10				
	16	39.60	4.25	5.23	3.72	4.03	4.26	5.21	9.47
	17	ND	2.37	7.57	5.14	3.95	3.04		
	18	54.89	6.18	8.59	3.05				
Ambient 1	19*	62.00	5.83	5.54	3.16				
	21	40.26	3.02						
	22	36.10	3.36	9.03	2.93				
Ambient 2	24*	5.33	4.40	7.59	4.14	3.19	1.26	0.09	0.12
	25	13.41	11.06	32.23	18.44	23.98	8.66		
	26	11.80	9.35	18.44	22.68	24.59	6.95	28.73	15.96
	28	8.03	7.59						
	29	16.51	9.38	33.12	29.47				
Ambient 3	31	14.49	9.82	16.68	8.03	13.42	11.52		
	32	24.00	5.82	30.66	20.27	28.34	12.77	13.25	8.97
	33	11.13	10.12						
	34	20.23	9.80	3.58	10.00				
	35*	16.11	3.82	7.76	3.73	1.90	0.79	0.92	ND

*Abiotic Pot ND none detation below detection limit

3.6 Total Ca Concentrations

To calculate the Ca release rate, it is necessary to add the Ca in the leachate with Ca found in the plant tissue (Equation 3). By the end of week 8, B_e experiments had released more Ca than B_a experiments (Figure 3-5). At week 8, the average Ca released from B_e experiments was 247 ± 67.65 µm and 136 ± 20.95 µm from B_a experiments (Appendix D). Despite this difference, a t-test found no significant difference between Be and Ba experiments in the total amounts of Ca released ($p>0.1$ Appendix H-4).

In both sets of abiotic experiments, weekly Ca concentration decreased throughout the experiment. Using a t-test to compare the average Ca concentrations each week, it was found there was not a significant difference between Ab_e and Ab_a ($p<0.05$). The average Ca released from Ab_e and Ab_a was 37.01 ±0.48 µmols and 28.18 ±0.37 µmols of Ca.

The presence of plants significantly increased the release of Ca ($p<0.05$ Appendix H-1). The B_e and B_a experiments released 81% and 66% more Ca than abiotic experiments, respectively (Appendix D).

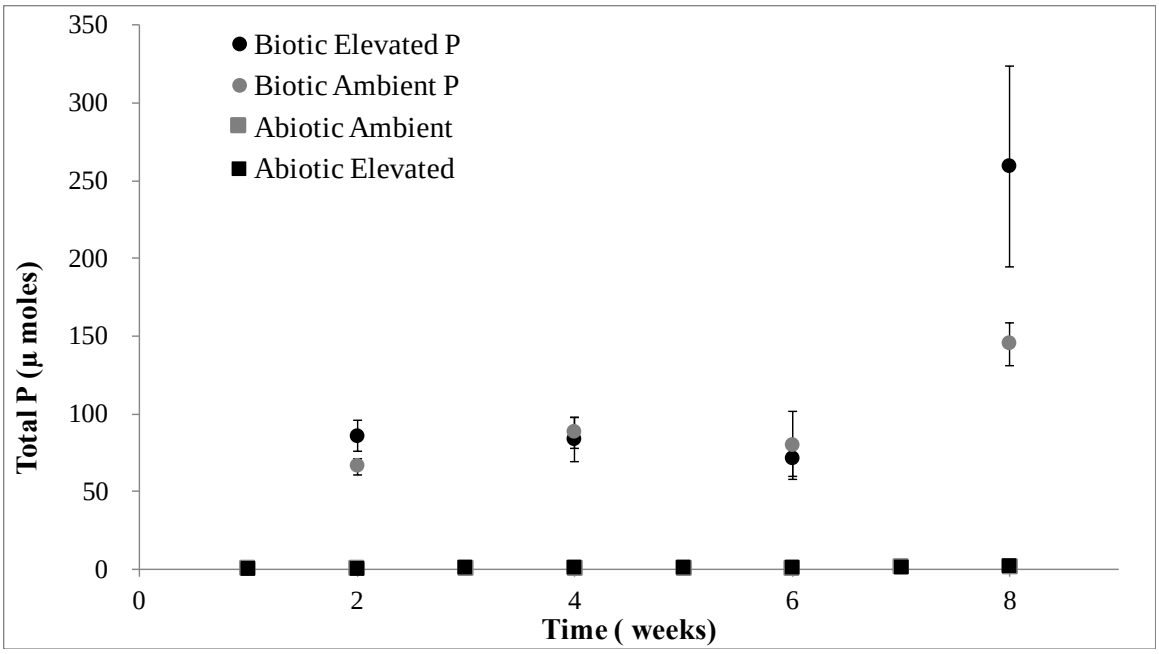


Figure 3-5. Concentrations of the total Ca released (µmoles) in each treatment. Total Ca includes Ca released in leachate as well as Ca contained in plant tissue.

3.7 Distribution of P pools

The majority of the liberated P was found in the plant tissue (Figure 3-6). Although only a small amount of P was measured in the leachate, it is still important to consider this pool for the following mass balance calculations. Plants grown in ambient CO_2 incorporated a higher percentage of P released from apatite than plants grown in elevated conditions.

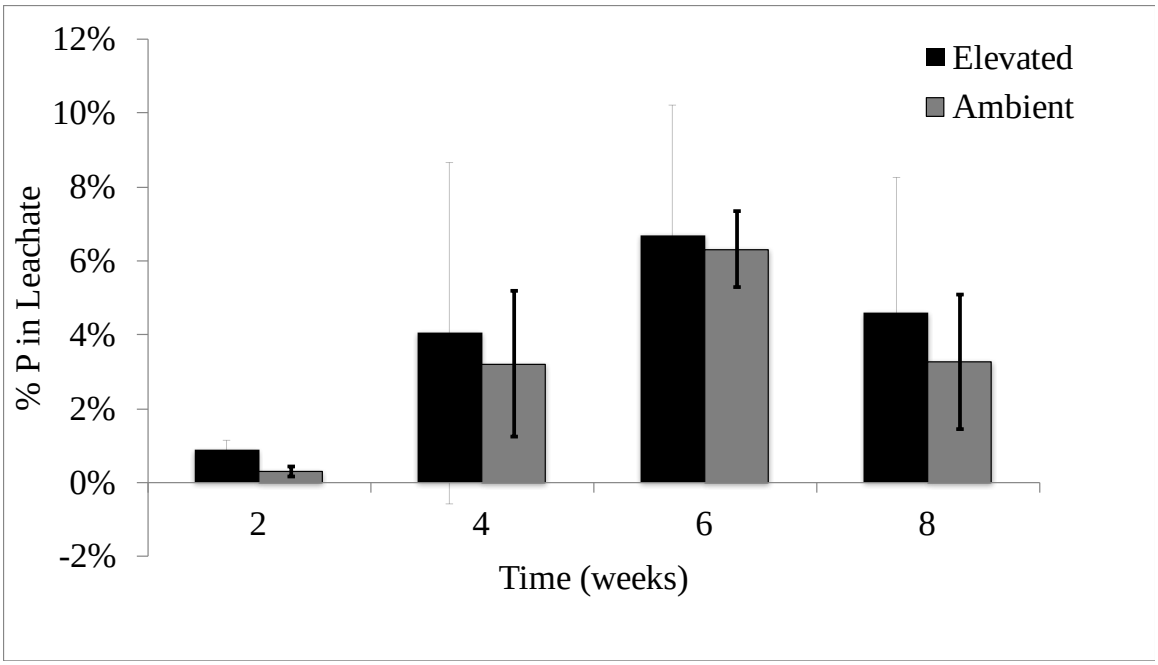


Figure 3-6. Distribution of P between plant tissue and leachate.

At week 3 the largest P concentration was measured in the leachate of B_e and B_a experiments. Following this increase, the concentration of P in solution steadily declined. Comparing the moving average of dissolved P from B_e and B_a experiments, it was found that significantly more P was released to solution by B_e experiments at weeks 2, 6 and 8 ($p<0.1$ Appendix H-5). Other weeks the concentrations were not significantly different.

The outlet solution of abiotic experiments shows P released from apatite also reached a maximum at week 3 and steadily decreases from that point (Table 3-6). The difference between concentrations of P released from the elevated and ambient sets of abiotic experiments wasn't significantly different at any point during the 8-week experiment ($p>0.05$).

Table 3-5. Moving average of P found in leachate.

	Biotic Elevated P (µmol)	Biotic Ambient P (µmol)	Abiotic Elevated P (µmol)	Abiotic Ambient P (µmol)
Week 1	0.68	0.42	0.05	0.12
Week 2	1.25	0.62	0.14	0.42
Week 3	2.89	2.33	0.72	0.57
Week 4	3.63	3.35	1.03	0.71
Week 5	4.14	3.31	1.11	0.83
Week 6	4.79	2.82	1.17	0.91
Week 7	5.25	2.85	1.39	0.93
Week 8	5.77	3.21	1.67	0.99

Table 3-6. P concentrations (µmol) measured in leachate during 8-week experiment.

Chamber No.	Column	Week 1 P (µmol)	Week 2 P (µmol)	Week 3 P (µmol)	Week 4 P (µmol)	Week 5 P (µmol)	Week 6 P (µmol)	Week 7 P (µmol)	Week 8 P (µmol)
Elevated 1	1*	0.005	0.070	0.409	0.223	0.022	0.026	0.188	0.014
	2	0.703	0.721	1.877	1.050	0.468	0.257	0.320	0.252
	3	0.112	0.264	0.919	0.360	0.137	0.609		
	5	0.773	0.120	0.405	0.246				
	6	0.096	0.231						
Elevated 2	1*	0.008	0.014	0.077	0.039	0.024	0.025	0.016	0.018
	8	0.000	0.032	0.387	0.087	0.157	0.061		
	9	0.063	0.046	0.118	0.070	0.136	0.084	0.065	0.057
	11	0.019	0.255						
	12	0.015	0.019	0.054	0.029				
Elevated 3	13*	0.038	0.002	0.081	0.038	0.032	0.011	0.016	0.240
	14	10.545	0.102						
	15	13.728	0.197	0.231	0.089				
	16	14.015	0.130	0.205	0.067	0.034	0.169	0.071	0.195
	17	11.909	0.138	0.546	0.242	0.047	0.098		
	18	9.830	0.162	0.587	0.199				
Ambient 1	19*	8.041	0.229	0.041	0.035				
	21	19.101	0.159						
	22	11.941	ND	0.169	0.087				
Ambient 2	24*	0.035	0.056	0.079	0.063	0.060	0.032	0.012	0.022
	25	0.330	0.116	1.648	0.431	0.453	0.048		
	26	0.184	0.303	0.865	0.579	0.192	0.034	ND	0.169
	28	0.002	0.016						
	29	0.485	ND	0.891	1.133				
Ambient 3	31	0.046	0.006	0.242	0.010	0.217	0.174		
	32	0.027	0.008	0.081	ND	0.156	0.028	0.022	0.067
	33	0.016	0.016						
	34	0.014	0.008	0.021	0.083				
	35*	0.041	0.009	0.032	0.036	0.016	0.020	0.007	0.015

*Abiotic Pot ND none detected

3.8 Total P Concentrations

To calculate the P release rate, it was necessary to combine dissolved P with P immobilized by the plant tissue (Equation 3). As shown in Figure 3-6, the majority of the released P was found in the plant tissue. At week 8 the average P released from Be experiments was 263.0 ± 58.2 µmoles and 145.8 ± 13.4 µmoles from Ba (Appendix D). Although the data suggest plants grown in elevated CO_2 released more P by the end of week 8, a t-test showed the averages were not significantly different ($p>0.1$ Appendix H-9).

The presence of plants significantly increased the release of P ($p<0.05$; Appendix H-2). The B_e and B_a experiments released 187 and 104 times more P than abiotic experiments by week 8.

In both sets of abiotic experiments, weekly P concentration decreased throughout the experiment. Using a t-test to compare the average P concentrations each week, it was found that there was not a significant difference between Ab_e and Ab_a ($p<0.05$). The average P released from Ab_e and Ab_a was 1.67 ±0.18 µmols and 0.99 ± 0.01 µmols of P respectively.

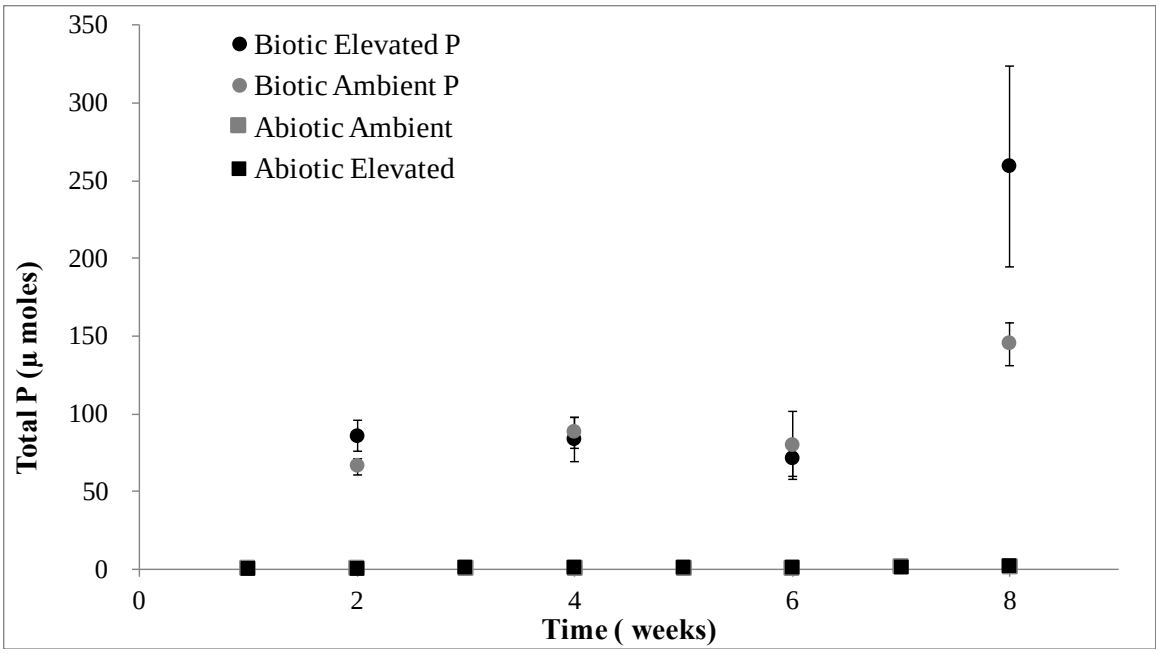


Figure 3-7. Total P (µmoles) released during 8-week experiment.

3.9 Stoichiometry of dissolution

Apatite used in experimentation had a Ca to P Ratio of 1.80. Examination of total P and Ca released shows dissolution was nonstoichiometric throughout all sets of experiments (Table 3-7). While biotic experiments released more P and had a Ca to P ratio below 1.80, abiotic experiments had a ratio above 1.80 and released a larger percentage of Ca. Although stoichiometric dissolution was never observed during the experiment, biotic experiments approached stoichiometric dissolution with time. The abiotic experiments had a Ca to P ratio which decreased with time. By the end of the experiment the ratio of Ca to P was still an order of magnitude higher than found in the apatite.

Batch reactor experiments designed to evaluate abiotic dissolution of apatite in the absence of interferences from quartz, had a Ca to P ratio ranging from 14.5 and 16.7 (Appendix F-4). Although batch reactor and column experiments aren't directly comparable, these two tests of abiotic dissolution suggest nonstoichiometric apatite dissolution was not solely due to adsorption of ions to quartz surfaces.

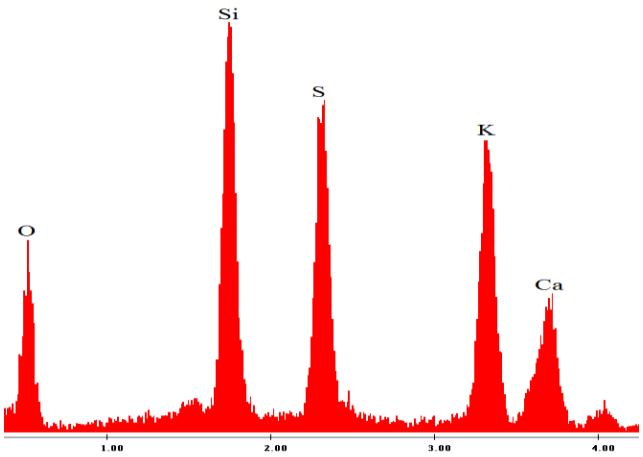
Table 3-7. Stoichiometry of Mineral dissolution

	Biotic Elevated	Biotic Ambient	Abiotic Elevated	Abiotic Ambient
Week 2	0.40	0.44	133.21	89.39
Week 4	0.63	0.87	25.86	36.40
Week 6	0.85	1.29	27.20	31.99
Week 8	1.11	0.94	23.31	29.61

3.10 SEM Characterization of Weathered Mineral Grains

In an effort to characterize the development of surface topographies and the formation of secondary precipitates, mineral grains collected from abiotic and biotic experiments were examined using the SEM. Grains from the biotic experiments were sampled from rhizosphere at each 2-week interval when plants were harvested. After rinsing and drying plant roots, apatite grains found adhered to root surface were also collected and examined separately. Although, precipitates were observed in both abiotic and biotic experiments

their compositions differed. EDS spectra of quartz grains from abiotic experiments



revealed precipitates rich in Ca and S (

Figure 3-10). In biotic experiments, precipitates on the surfaces of quartz grains contained Fe, Al and P (Figure 3-8). Apatite grains removed from the root surface showed the development of needle like structures similar to those described by Calvaruso et al. (2013) and Dorozhkin (1997) (Figure 3-9). While grains removed directly from the root surface had developed easily observed surface topographies, examination of grains recovered from the rhizosphere did not reveal formation of needles. It is likely formation of needles increased the surface area of apatite during the experiment.

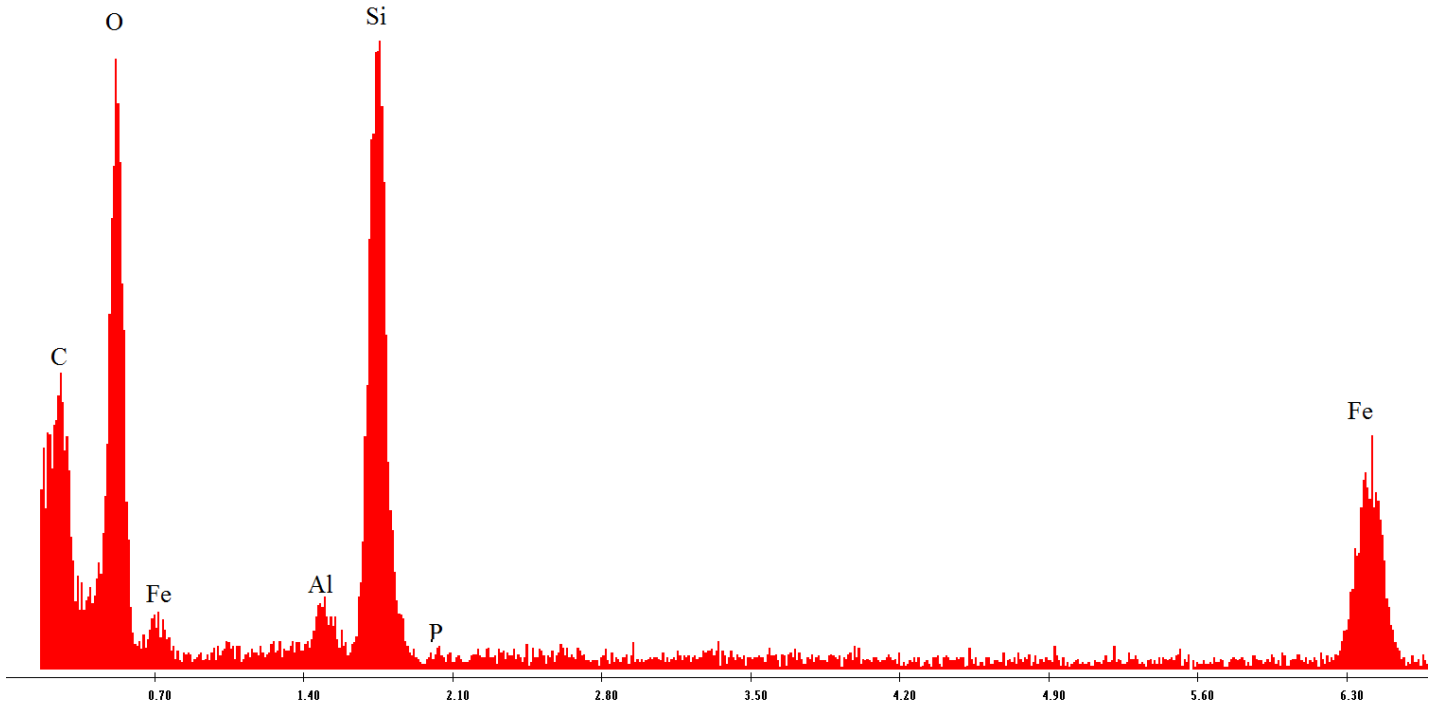


Figure 3-8. EDS spectra of precipitates on the surface of quartz grains in biotic experiments. Concentrations of P show that incongruent dissolution can be partially accounted for by the formation of precipitates.

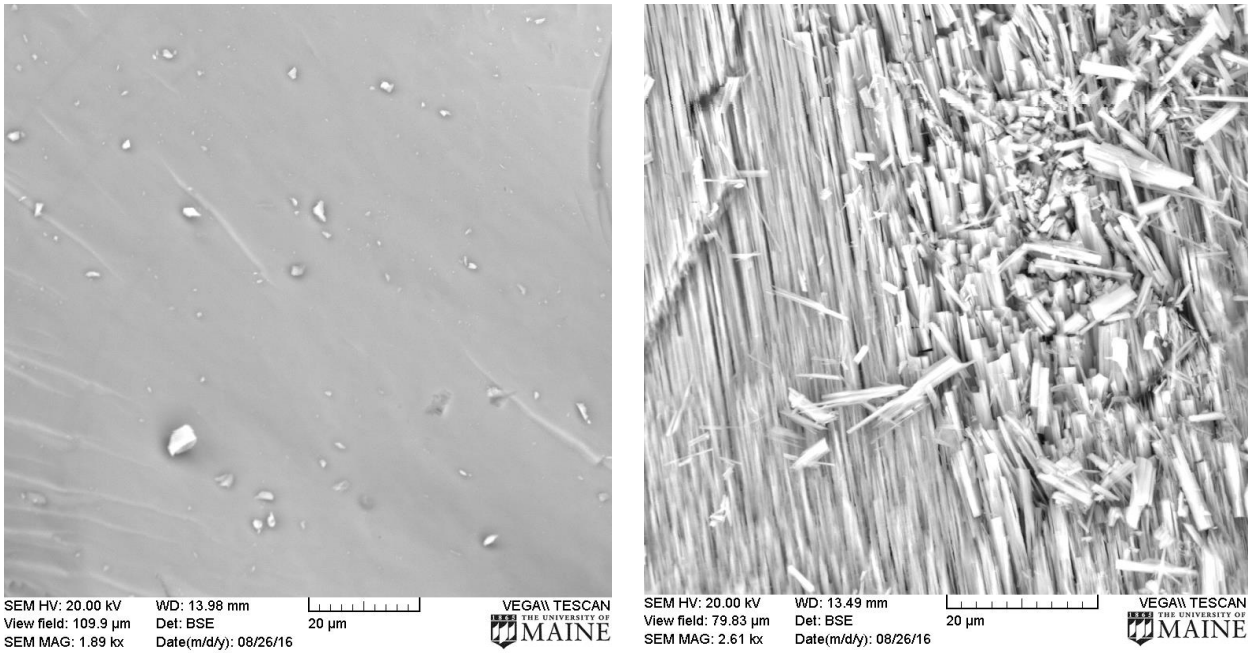


Figure 3-9. Photomicrograph of cleaned and unweathered apatite surface (left) and apatite surface after 8 weeks on the surface of a root (right). The images shown represent the extremes of surfaces.

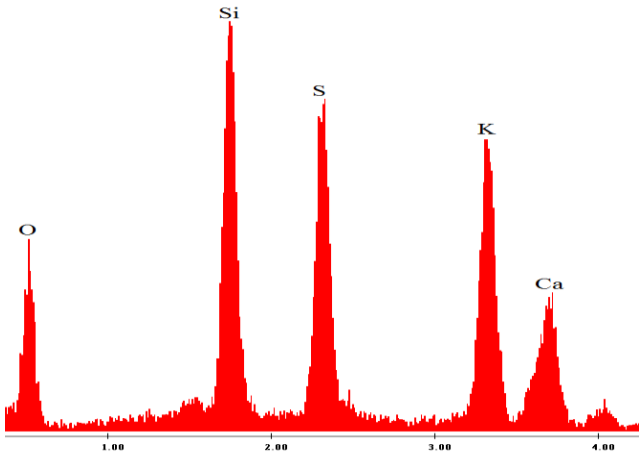


Figure 3-10. EDS spectra of precipitates found in abiotic experiments.

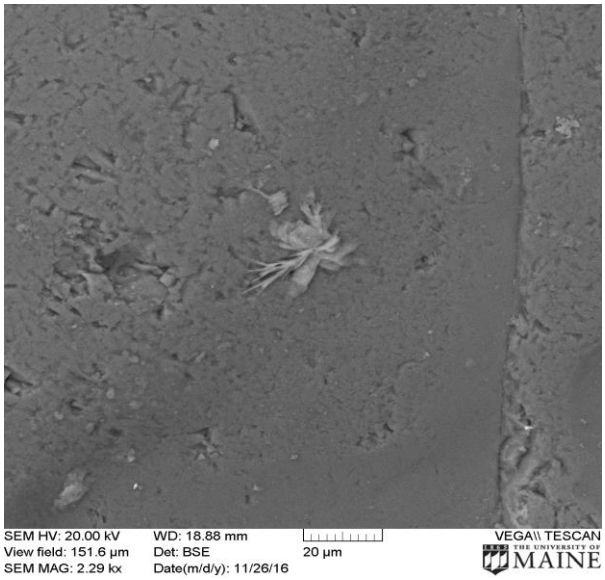


Figure 3-11. Photomicrograph of Ca sulfate precipitate formed on the surface of a quartz grain recovered from biotic experiment.

4. DISCUSSION

Numerous studies show that elevated CO_2 increases carbon allocation to the rhizosphere (Rao et al., 2015; Haase et al., 2007; Salsman et al., 1999 Poorter, 1993). This study seeks to understand one possible change brought about by increases in below ground carbon fluxes caused by higher pCO_2. Using flow-through microcosms we have collected data suggesting an increase in mineral weathering due to plants being grown in elevated CO_2.

In response to a higher nutrient demand and more available C in the air, plants undergo morphological changes. Work by Huber (1989) and Lin et al. (2000) showed that elevated atmospheric CO_2 causes an increase in the root to shoot ratio of plants. This was also observed in our study (Figure 3-2). Increased root growth has been shown to improve nutrient uptake by increasing the surface area of roots and aiding in the acquisition of nutrients (Wang et al., 2009). Larger quantities of below ground tissues create observable changes to rhizosphere chemistry and intensifies chemical weathering.

4.1 Relationship Between pH and Ion Release

Apatite dissolution is strongly associated with solution pH. In low pH solutions, apatite dissolves via the following reaction (Bengtsson & Sjöberg 2009):

$$Ca_5(PO_4)_3(F,OH) + 7H^+ = 5Ca^{2+} + 3H_2PO_4^- + H^+ + F^-,H_2O$$

Although this formula includes the consumption of protons, pH measurements in some of the experiments in this study fell (Figure 3-1). The increased acidity seen in biotic experiments is due to several different processes. Plants need to maintain an

internal pH and charge balance. If there is an excess of cations is taken up, the plant root compensates by releasing the surplus positive charges as protons (i.e., ion-exchange reaction), acidifying the rhizosphere. The same process occurs for consumption of anion nutrients which results in the release of OH^- (Hinsinger et al., 2003). Decreases in pH are also attributed to the release of organic acids, which are derived in the roots during cellular respiration. The importance of organic acids in changing soil solution pH is highly variable between plant species and difficult to study due to their quick consumption by microbes (Ryan et al., 2001).

Some pH decrease can also be attributed to the production of carbonic acid ($CO_{2(aq)}$). Roots generate energy from respiration, releasing CO_2 into the rhizosphere which forms carbonic acid. However, contribution to acidity by carbonic acid would be small in the observed pH (Appendix A). In a review by Hinsinger et al. (2003) a large range of soil CO_2 values are reported. These differences arise from variability of soil structure, microbial respiration, and soil organic matter. Helal & Sauerbeck (1989) found that up to 15% of all photosynthetically fixed carbon in maize plants ends up below ground as CO_2. These quantities of respired CO_2 are nearly an order of magnitude higher than concentrations of H^+ due to cation exchange.

EDS spectra of grains from abiotic experiments reveal areas with high concentrations of C indicating the possible presence of unwanted microbial populations (Appendix I). It is possible that some of the decrease in pH can be attributed to the growth of microbes,

which acidify their surroundings through respiration and the consumption of cation nutrients.

Calcium and P concentrations in solution increase with decreasing pH due to enhanced mineral dissolution. The differences between Ca and P concentrations in the leachate in B_e and B_a were not significant until there was a significant difference in pH between the two treatments. This is not surprising since apatite dissolution shows a strong pH dependence (Bengtsson & Sjöberg 2009; Chaïrat et al., 2007). At week 5 the pH values in B_e columns were significantly lower than B_a columns (Appendix H-7). Following week five, the Ca released into the leachate of B_e columns was significantly higher (Figure 7). With respect to total Ca released (sum of effluent and plant tissue concentrations) the differences between B_e and B_a were not significant until week 8. This could be attributed to the slower response time from plant tissue, the timing of sample collection and the much smaller concentration of Ca captured by plant tissue (Figure 3-5).

There was a similar response to decreased pH and the release of P. The differences in P concentrations between Be and Ba experiments were greatest when there was a significant difference in average pH. At week 5, the pH of Be experiments was significantly lower than that in Ba experiments. Interestingly, leachate P concentrations in the Be experiments increased, while they decreased in the Ba experiments (

). During week 7, the pH of the Ba experiments reached a maximum at 5.60 and concentrations of P in the leachate were lower than any other point during the experiment. At that time the pH and P concentrations of Ba experiments resembled those

in the abiotic experiments (Figure 3-1). Similar to the Ca data, changes in P release are not reflected in plant tissue concentrations until week 8 (Figure 3-7). Although the mean P concentration of Be experiments was greater than Ba plants, the difference was not significant. This could be attributed to the small sample size (n=2).

4.2 Calculated weathering rates

Weathering rates for biotic experiments were calculated based Equation 5 (Calvaruso et al. 2013). Quantification of total Ca and P allowed us to calculate two separate dissolution rates for biotic and abiotic experiments (Table 11, Table 12). Comparing the dissolution rates of biotic experiments based on Ca and P concentrations using a t-test, it was found that there was not a significant difference between the two rates on any of the four sampling dates ($p>0.05$, Appendix H-12).

Because dissolution was far from stoichiometric in abiotic experiments (Table 3-7), dissolution rates calculated using Ca and P varied by two orders of magnitude. Possible explanations for this difference are explored in greater detail in the following section.

4.2.1 Dissolution rates based on Ca concentrations

The concentrations of Ca released from the Ab_e and Ab_a experiments show increased CO_2 concentrations did not lead to a significant change in Ca release (Table 3-4). Because of this, data from Ab_e and Ab_a were combined to calculate one average dissolution rate based on Ca concentrations, which decreased with time (Table 4-1)

In both sets of biotic experiments, the dissolution rates were fastest at week 2, when the mineral surfaces were fresh. In B_a experiments the apatite dissolution rate gradually decreased for the remaining 6 weeks of the experiment. In the B_e experiments the rate decreased until week 6, when the average rate increased from $10^{-12.06}$ to $10^{-11.86}$ mol cm^2 s^{-1} (Table 4-1). This increase in weathering rate coincides with the precipitous drop in pH observed in B_e columns (Figure 3-1). Although the average rate in biotic elevated experiments was faster than biotic ambient experiments, a t-test shows the difference was not significant ($p>0.05$; Appendix H-10).

Table 4-1. Apatite dissolution rates based on concentrations of Ca. Release rates are shown as log (mol $cm^{-2}sec^{-1}$).

	Biotic Elevated	**Biotic Ambient**	**Abiotic**
Week	Log (mol $cm^{-2}sec^{-1}$)	Log (mol $cm^{-2}sec^{-1}$)	Log (mol $cm^{-2}sec^{-1}$)
Week 2	-11.73	-11.59	-12.25
Week 4	-11.96	-11.95	-12.35
Week 6	-12.06	-12.06	-12.44
Week 8	-11.86	-12.08	-12.52

4.2.2 Dissolution rates based on P concentrations

Because Ca and P release rates were non-stoichiometric, dissolution rates were also calculated using total released P. Previous studies have successfully used dissolved P in solution to quantify apatite dissolution (Welch et al. 2002). In experiments conducted by Calvaruso et al. (2013), the release of P was determined to represent the true dissolution rate of apatite due to the formation of Ca precipitates observed on the surface of mineral

grains. In our experiments, the ratio of Ca to P in biotic experiments also shows possibility of Ca loss to precipitates (Figure 3-11).

The two dissolution rates calculated based on Ca and P concentrations in biotic experiments follow a similar trend throughout the duration of experiments (Figure 4-1). As seen in the dissolution rates based on Ca concentrations, the dissolution rate in B_e experiments increased between weeks 6 and 8. This increase in the dissolution rate occurs after the pH falls in B_e experiments (Figure 3-1). Although the average B_e dissolution rate is greater than the B_a rate, a t-test demonstrated there was no significant difference between rates in the two treatments ($p>0.05$ Appendix H-11).

As mentioned previously, the dissolution rates for abiotic experiments differed greatly when calculated based on Ca and P concentrations. When the abiotic rate was calculated based on P concentrations, the dissolution rate was two orders of magnitude slower than the rate based on Ca concentrations. This difference in dissolution rates is explored in greater detail in the following section.

The presence of plants caused P to be released approximately 100-1000× faster than abiotic treatments (Table 4-2). The greatest difference in dissolution occurred at week 2 when mineral surfaces were fresh.

Table 4-2. Apatite dissolution rates calculated based on concentration of P. Release rates are shown as mol cm^{-2} s^{-1}.

Week	**Biotic Elevated** Log (mol $cm^{-2}sec^{-1}$)	**Biotic Ambient** Log (mol $cm^{-2}sec^{-1}$)	**Abiotic** Log (mol $cm^{-2}sec^{-1}$)
Week 2	-11.65	-11.77	-14.46
Week 4	-12.05	-12.10	-13.95
Week 6	-12.30	-12.26	-14.07
Week 8	-11.93	-12.15	-14.04

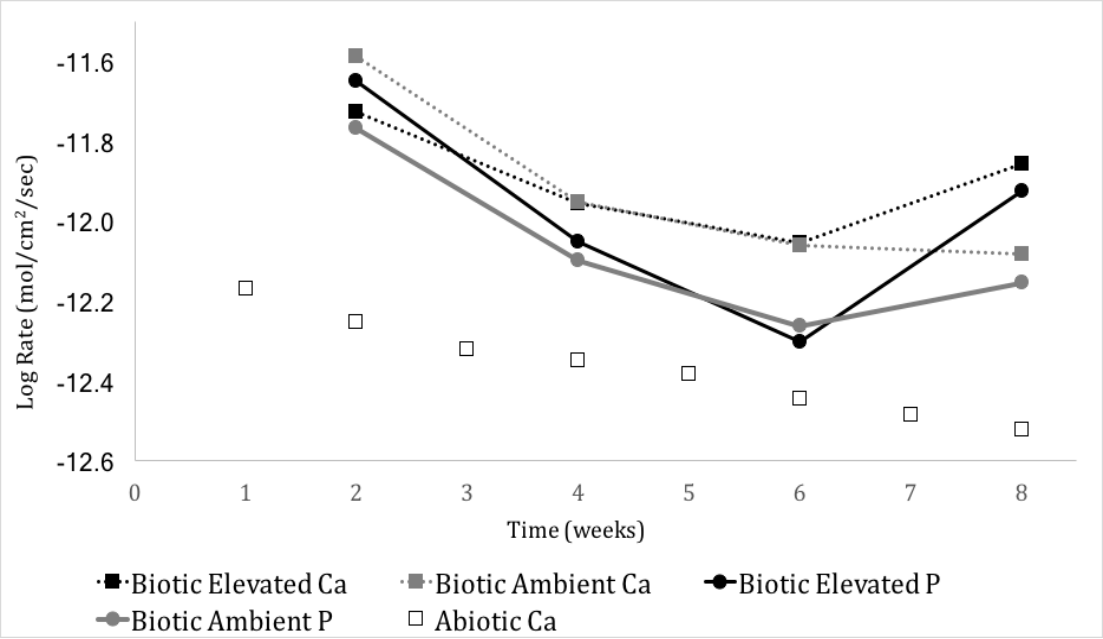


Figure 4-1. Rates of apatite dissolution based on Ca and P release log(mol cm^{-2} sec^{-1}). Dissolution rate based on P release not included because it is two orders of magnitude slower than other rates.

4.3 Rates measured in other apatite dissolution studies

There are many other studies which have sought to determine the dissolution rate of apatite. Most studies focus on effects of pH and synthesized organic ligands. Welch et al. (2002) did a series of batch reactor studies using inorganic acids in pH ranging from 2-6 and found dissolution rates ranging from 10^{-9} mol cm $^{-2}$ s^{-1} at the minimum pH and 5×10^{-13} mol $cm^{-2}s^{-1}$ at the highest pH. This is comparable to Valsami-Jones et al. (1998), who used a pH range of 4 to 5.2 and calculated a dissolution rate based on Ca of 1.8 to 3×10^{-11} mol $cm^{-2}s^{-1}$. when looking at the effect of organic ligands (oxalate and acetate) on apatite dissolution, Welch et al. (2002) found the presence of these ligands increased dissolution rates by two orders of magnitude but chose not to share the actual rates. They also noted the effects of organic ligands became less pronounced at lower pH as the solution pH was approaching the pK_a of oxalate, 3.19 and acetate, 1.18. Hutchens et al. (2006) compared abiotic apatite dissolution to apatite dissolution in the presence of bacteria. They report a rate of 2.63×10^{-15} mol $cm^{-2}s^{-1}$ for abiotic experiments and 2.37×10^{-14} mol $cm^{-2}s^{-1}$for biotic experiments.

There are very few studies which seek to measure apatite dissolution in the presence of plants. Calvaruso et al. (2013) conducted apatite dissolution studies using scots pine grown in flow through column reactors. The presence of plants increased dissolution from 1.8×10^{-14} to 1.72×10^{-13} mol $cm^{-2}s^{-1}$.

In our experiments, the rate of dissolution for abiotic experiments based on Ca release was initially $5.59\times10^{-13} \pm 3.3\times10^{-14}$ mol $cm^{-2}s^{-1}$and slowed to a rate of $2.99\times10^{-13} \pm$

4.4×10^{-15} mol $cm^{-2}s^{-1}$ by week 8. In our biotic experiments rates were closer to those created by the use inorganic acids and were on the order of 10^{-12} mol $cm^{-2}s^{-1}$

4.4 Stoichiometry of Apatite weathering

Congruent dissolution of typical apatite results in a Ca to P ratio of approximately 1.67. Apatite used in our experiments had a Ca to P ratio of ~1.80 (Table 3-1). All abiotic experiments resulted in release ratios that were significantly higher than what is typically found in stoichiometric apatite dissolution (Table 3-7). The unexpectedly low concentrations of P in abiotic effluents could be due to the highly reactive nature of P. Fe and Al precipitates were observed in EDS spectra of quartz grains from abiotic columns. The presence of Al further indicates the possible presence and weathering of feldspar grains mentioned in the materials and methods section. Grains were not heavily coated but patches of precipitates were easily found (Appendix I-1). Some of the observed precipitates included P. This suggests that some P could have been lost due to the formation of iron or aluminum phosphates. Arias et al. (2006) observed that Fe oxides in packed quartz sand filters were able to remove up to 50% of total P from natural and waste waters.

Batch reactor experiments of apatite grains weathered with the nutrient solution used for plant growth achieved a Ca to P ratio that was closer to the stoichiometry but was still an order of magnitude higher than 1.80 (Appendix F-4). These experiments show the adsorption of P to Fe and Al oxides on quartz grains was not the sole reason for a higher Ca to P ratio. It is also possible P was released from apatite but readsorbed to apatite

surface. Dissolution experiments by Chaïrat et al. (2007); Guidry & Mackenzie (2003) and Valsami-Jones et al. (1998) observed dissolution rates based on Ca concentration to be nonlinear in neutral pH conditions and initially incongruent. This break in linear dissolution rates has been shown to be correlated with concentration of $CaOH_2^+$ in solution.

Incongruent dissolution occurs due to two separate locations of Ca in the crystal structure of apatite. The first Ca site consists of four Ca atoms in nine-fold coordination and the second site consists of six Ca atoms in seven-fold coordination. Both of these Ca polyhedra are connected to the phosphate tetrahedra. Before any P can be released into solution all bonds between calcium and oxygen must be broken. Because of this, formation of non-stoichiometric surface layers have been observed in experiments by Brown & Martin, (1999) and Park et al. (2005).

In biotic experiments the ratio of Ca to P released was lower than the expected stoichiometric ratio of apatite (Table 3-7). SEM analysis of quartz grains from the rhizosphere of biotic columns reveal the presence of Ca sulfate precipitates (Figure 3-11). These precipitates were easily found, but were sporadically distributed along the surface of quartz grains (Appendix I).

4.5 Nutritional Status of Plants

Standard nutrient concentrations and visual diagnostic aids for assessing nutritional status of plants are abundant in books by Benton et al., (1996) Gauch (1972), and Martin (1979). Large variations in typical nutrient concentrations exist due to plant type,

sampled tissue, and stage of development. Because of these uncertainties, an analysis of total elemental concentration works best in chorus with visual identification of nutrient deficiencies. Elemental analysis of plants grown in both elevated and ambient conditions have lower concentrations of Zn, Ca and (

Table 3-3) than standard concentrations found in leaves of healthy bean plants collected by Benton et al. (1996). As a micronutrient, Zn is required in smaller concentrations than other nutrients. Zn deficiency causes a decrease in auxin, the protein required for elongating cells in shoots and regulating plant growth. In the absence of Zn, auxin oxidizes more quickly and plants produce smaller leaves and suffer reductions in shoot length. Some plants were clearly stunted while others appeared healthy (Appendix I-2). Hewett and Smith (1974) observed 13-20 μg of Zn in *P. vulgaris* seeds. It is possible this helped sustain healthy plant growth was sustained for most of the experiment. Studies by Park et al. (2005) have shown trace concentrations of Zn in the structure of apatite. Although most plants survived the 8-week duration of the experiment, plant fatalities could be reduced by adding a $ZnSO_4$ component which is a common in other nutrient solutions (Hewitt & Smith, 1974). While concentrations of Ca in the outlet solution were high, samples of plant tissue were deficient in Ca (

unnecessary spacing

Table 3-3). It is possible that Ca uptake was impaired by high soil moisture which disrupts the mechanism for Ca uptake, as well as by the virtually nonexistent CEC of

quartz sands used in experiments. Without any adsorption capacity, Ca would be quickly lost from the plant pots from the movement of water (Martin, 1979; Mclaughlin & Wimmer, 1999).

4.6 Real World Implications of In-vitro Studies

Although these data are novel, there are several unrealistic conditions imposed by the experimental design. It is important to remember that these data were collected in a laboratory setting with an approximate pCO_2 of 1000 ppm. This is around 200 ppm higher than any model of future atmospheric CO_2 suggests (Girod et al., 2009). Additionally, there is no organic component to the soil used in this experiment. Organic material greatly influences P movement through adsorption and immobilization by organic matter. This P pool can represent up to 80% of P in soils (Hinsinger, 2001; Jungk & Claassen, 1989; Martin, 1979). Without these sinks to strip P from the soil solution, concentrations of P in the leachate of our experiments was roughly 4 orders of magnitude higher than typical concentrations outlined by Richardson (1994).

The inorganic component of soil P was also atypical. Natural soils are estimated to have inorganic P concentrations that range between 0.005-0.02 P g cm^{-3} in the top 10 cm. The inorganic P is present as phosphate minerals, Fe, Ca and Al precipitates and adsorbed to mineral surfaces. In our experiments, the only P source was the 15 g of apatite added to each reactor column. Using the volume and percent weight P of apatite, a value of 0.04 g cm^{-3} P was calculated.

Rates of mineral dissolution show a great deal of variability between different labs and when comparing in situ studies to in vivo studies. Factors such as mineral stoichiometry, heterogeneity, preparation, and experimental conditions and durations affect dissolution rates (Dorozhkin, 2012). Comparisons between laboratory and field studies have shown mineral weathering in lab settings to occur at three to five orders of magnitude faster than in field settings (Maher et al., 2006). These dissimilarities arise from differences in surface areas, formation of precipitates, approach to saturation, mineral composition and dominant flow paths (Drever, 2005; Maher, 2010). Putting these large differences between in vitro and in vivo studies aside, these are some of the first data indicating plants exposed to elevated CO_2 are better able to release nutrients from mineral sources (Andrews, Leake, Palmer, Banwart, & Beerling, 2011; Fransson & Johansson, 2010; Williams, Walter, Ku, Kling, & Zak, 2003). Increases in acidity, organic ligands and root surface area, like those seen in this study, are all traits that aid in nutrient acquisition from inorganic sources.

5. CONCLUSIONS

The presence of *P. vulgaris* increases the rate of apatite dissolution. Increases in dissolution rates translate to a larger concentration of bioavailable nutrients, specifically Ca and P. This study shows that the presence of *P. vulgaris* released Ca from apatite 1.32-1.45 times faster than abiotic controls. More importantly, experiments containing plants exhibited a rate of P release of 118-152 times the abiotic controls (Appendix G-2). In both biotic and abiotic experiments, apatite dissolution was nonstoichiometric. In biotic experiments, P, an essential component of DNA and ATP, was released in greater quantities than Ca. In the absence of plants, the quantity of P released was minimal. Previous studies show apatite dissolution is pH dependent and can be enhanced by the presence of organic acids. Our data suggest apatite dissolution was promoted by a mixture of cation exchange reactions, evident by the decrease in pH, and complexation reactions created by organic ligands seen by the increase in DOC.

Root mass and acidification of the rhizosphere increased in experiments where plants were grown in elevated CO_2. These changes to rhizosphere surface area and chemistry resulted in higher concentrations of Ca and P found in plant tissue and the outlet solution. Although these differences between rates and total Ca and P are statistically insignificant for most points during the experiment, larger sample sizes could eliminate uncertainties related to variance in plant growth (appendix H-11). Leachate data had a larger sample size and was more homogeneous ion concentrations, which allowed t-tests that show the means were different. At weeks 2, 4 and 8, concentrations of Ca in the leachate from B_e experiments were significantly higher than those in B_a experiments (Appendix H-4).

Additionally, P concentrations in the leachate from B_e columns were significantly higher at weeks 2, 6 and 8 than B_a experiments (Appendix H-5).

5.1 Potential Implications

Nutrient availability greatly limits production in both fertilized and unfertilized ecosystems. In particular, P, which is an essential element in both heterotrophs and autotrophs, limits production in roughly 2/3 of the cultivated soil on earth (Batjes, 1997). Without P, energy transfer in cells is impossible. In recent years there is growing concern regarding longevity of P reserves at Earth's surface. P derived from phosphate rock is mined for fertilizer production and different estimates show these deposits could be depleted within the next 50-100 years (Cordell et al., 2009). Understanding P dynamics and possible increases in P demands of crop species are at the center of ensuring food security in our rapidly changing world.

Natural ecosystems are also undergoing changes due to anthropogenic increases in CO_2. As global temperatures rise, environments that were once too cold to support autotrophs and develop organic soils can now be colonized. Studies of paleoecosystems and contemporary forests show plant communities migrating to higher altitudes and latitudes during warmer periods (Jump et al., 2009; Overpeck et al., 1991; Woodall et al., 2009). Some studies have highlighted the potential for forest ecosystems being outpaced by climactic changes (Schwartz et al., 2001). Higher altitudes and latitudes that were once dominated by glaciers produce large quantities of till, which have a large surface area and have previously undergone minimal chemical weathering. These labile nutrient

sources combined with increased belowground C fluxes have the potential to shape the rate at which plants are able to colonize newly available environments. Quantifying increases in plant-driven nutrient release from mineral sources in elevated CO_2 is an important parameter for making accurate predictions about soil formation rates, nutrient release, and subsequent plant migration.

5. 2 Future Work

Because of the wide array of biotic and abiotic processes taking place in soils, their effects on nutrients are difficult to accurately quantify and follow in the field, especially on short timescales (Williams et al., 2003). Nevertheless, predicting and understanding changes to these cycles will help ensure continued use of the Earth's fragile surface. Previous work has looked at plant-mineral weathering through the lens of plant sciences, and data mostly focus on quantifying nutrient fluxes between soils and plants rather than mineral dissolution rates. To my knowledge, there are no studies comparing mineral dissolution rates between plants grown in ambient CO_2 and elevated CO_2. Understanding the response of plants grown in elevated CO_2 and subsequent mineral weathering is an important endeavor for making predictions about soil formation and nutrient loss as well as discover new forms of bioremediation.

These experiments represent a successful test of our experimental design in a laboratory setting. The CO_2 control system could easily be moved to a greenhouse or an outdoor research plot. For a variety of reasons explored in the discussion, data from these microcosm studies are just the beginning of investigating changes brought about by

increases in C fluxes caused by plant growth in elevated CO_2. Due to the ever shrinking quantities of P sources, particular interest should be aimed at understanding changes in P dynamics in soil systems.

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Appendix A Flow rate and pH

Table A-1 Week 1 pH, Volume and Time

None Detected (ND) Not Sampled (NS) Volume (V) Time(t)

Week 1	*Abiotic Elevated*		
Sample ID	*pH*	*V (g)*	*t (min)*
1	5.02	45.91	10080
7	4.93	35.69	
13	4.90	34.29	
Week 1	*Abiotic Ambient*		
Sample ID	*pH*	*V (g)*	*t (min)*
19	3.59	117.85	
24	4.81	12.61	
35	5.94	56.93	
Week 1	*Biotic Elevated*		
Sample ID	*pH*	*V (g)*	*t (min)*
2	4.29	101.68	
3	4.58	114.28	
4	4.54	109.11	
5	4.35	128.65	
6	5.47	118.19	
8	5.16	92.30	
9	5.34	97.98	
10	5.49	115.66	
11	5.31	104.30	
12	5.28	95.32	
14	3.92	88.55	
15	3.00	89.77	
16	3.67	114.01	
17	3.57	101.30	
18	3.59	113.59	
Week 1	*Biotic Ambient*		
Sample ID	*pH*	*V (g)*	*t (min)*
21	3.87	124.90	
22	4.07	98.83	
23	3.86	119.45	
25	4.62	34.05	
26	5.06	47.70	
27	5.02	37.32	
28	4.97	40.73	
29	5.01	29.21	
30	5.72	82.55	
31	6.44	109.02	
32	5.86	111.54	
33	5.86	106.62	
34	5.96	97.26	

Table A-2. Week 2 pH, flow rate (g), time (min) and water content of harvested plant pot (g).

None Detected (ND) Not Sampled (NS) Volume (V) Time (t)

Week 2	*Abiotic Elevated*			
Sample ID	*pH*	*V (g)*	*t (min)*	*Water Content (mL)*
1	6.00	40.89	17790	NS
7	4.89	29.54		NS
13	4.98	13.37		NS
Week 2	*Abiotic Ambient*			
Sample ID	*pH*	*V (g)*	*t (min)*	*Water Content (mL)*
19	4.50	89.60		NS
24	4.68	42.15		NS
35	5.78	58.67		NS
Week 2	*Biotic Elevated*			
Sample ID	*pH*	*V (g)*	*t (min)*	*Water Content (mL)*
2	4.43	97.75		NS
3	5.24	87.38		NS
4	4.26	85.76		NS
5	4.57	64.71		NS
6	5.03	56.32		9.79
8	5.06	86.40		NS
9	5.00	59.59		NS
10	5.36	88.72		NS
11	5.05	53.49		8.36
12	5.29	84.70		NS
14	4.77	68.69		6.84
15	4.52	79.73		NS
16	4.63	83.79		NS
17	4.47	59.34		NS
18	4.52	71.46		NS
Week 2	*Biotic Ambient*			
Sample ID	*pH*	*V (g)*	*t (min)*	*Water Content (mL)*
21	4.51	92.01		8.67
22	4.45	76.98		NS
23	4.72	101.89		NS
25	4.59	106.04		NS
26	4.71	99.90		NS
27	4.79	116.85		NS
28	4.84	116.75		9.08
29	4.73	81.51		NS
30	5.16	64.53		NS
31	5.17	85.30		NS
32	5.70	89.54		NS
33	5.16	74.15		7.57
34	5.75	80.20		NS

Table A-3. Week 3 pH, volume (g) and time (min)

None Detected (ND) Not Sampled (NS) Volume (V) Time (t)

Week 3	*Abiotic Elevated*		
Sample ID	*pH*	*V (g)*	*t (min)*
1	5.23	110.24	31590
7	4.88	149.98	
13	5.32	172.33	
Week 3	*Abiotic Ambient*		
Sample ID	*pH*	*V (g)*	*t (min)*
19	4.54	117.46	
24	4.82	174.16	
35	5.53	153.01	
Week 3	*Biotic Elevated*		
Sample ID	*pH*	*V (g)*	*t (min)*
2	4.57	151.29	
3	4.82	126.63	
4	6.36	127.92	
5	5.10	96.77	
6	NS	NS	
8	4.47	92.71	
9	4.55	36.72	
10	5.13	164.68	
11	NS	NS	
12	5.19	113.41	
14	NS	NS	
15	4.91	183.85	
16	4.88	203.35	
17	4.63	207.80	
18	4.56	169.23	
Week 3	*Biotic Ambient*		
Sample ID	*pH*	*V (g)*	*t (min)*
21	NS	NS	
22	4.60	83.68	
25	4.53	115.32	
26	4.60	107.04	
29	4.48	123.22	
30	NS	NS	
31	4.80	110.61	
32	5.33	66.39	
33	NS	NS	
34	5.47	32.08	

Table A-4. Week 4 pH, volume (g), time (min) and water content of harvested plant pot (g).

None Detected (ND) Not Sampled (NS) Volume (V) Time (t)

Week 4	*Abiotic Elevated*			
Sample ID	*pH*	*V (g)*	*t (min)*	*Water Content (mL)*
1	5.66	107.13	40470	NS
7	4.89	117.26		NS
13	4.57	121.23		NS
Week 4	*Abiotic Ambient*			
Sample ID	*pH*	*V (g)*	*t (min)*	*Water Content (mL)*
19	4.40	86.81		NS
24	4.92	126.05		NS
35	5.09	113.56		NS
Week 4	*Biotic Elevated*			
Sample ID	*pH*	*V (g)*	*t (min)*	*Water Content (mL)*
2	5.68	109.03		NS
3	5.40	101.26		NS
4	NS	NS		NS
5	6.20	92.31		NS
6	NS	NS		NS
8	3.80	27.94		NS
9	4.29	13.28		NS
12	4.64	89.08		10.44
14	NS	NS		NS
15	4.65	120.70		NS
16	4.83	113.23		NS
17	4.98	117.91		NS
18	4.69	104.11		7.59
Week 4	*Biotic Ambient*			
Sample ID	*pH*	*V (g)*	*t (min)*	*Water Content (mL)*
21	NS	NS		NS
22	4.38	62.10		8.91
23	NS	NS		NS
25	4.67	54.76		NS
26	4.50	72.77		NS
29	4.47	112.64		11.71
30	NS	NS		NS
31	4.71	76.96		NS
32	5.10	31.83		NS
33	NS	NS		NS
34	5.52	106.83		11.18

Table A-5. Week 5 pH, volume (g) and time (min)

None Detected (ND) Not Sampled (NS) Volume (V)Time(t)

Week 5	*Abiotic Elevated*		
Sample ID	*pH*	*V (g)*	*t (min)*
1	5.04	109.55	50430
7	4.88	142.84	
13	4.96	109.90	
Week 5	*Abiotic Ambient*		
Sample ID	*pH*	*V (g)*	*t (min)*
19			
24	5.81	108.81	
35	5.19	128.03	
Week 5	*Biotic Elevated*		
Sample ID	*pH*	*V (g)*	*t (min)*
2	4.67	100.73	
3	479.00	88.07	
4	NS	NS	
5	NS	NS	
6	NS	NS	
8	3.87	135.64	
9	4.30	20.67	
15	NS	NS	
16	4.91	100.68	
17	4.90	108.45	
18	NS	NS	
Week 5	*Biotic Ambient*		
Sample ID	*pH*	*V (g)*	*t (min)*
21	NS	NS	
22	NS	NS	
23	NS	NS	
25	4.80	136.36	
26	5.51	152.24	
27	NS	NS	
28	NS	NS	
29	NS	NS	
30	NS	NS	
31	4.78	68.04	
32	5.04	133.94	
33	NS	NS	
34	NS	NS	

Table A-6. Week 6 pH, volume (g) time (min) and water content of harvested plant (g)

None Detected (ND) Not Sampled (NS) Volume (V) Time (t)

Week 6	*Abiotic Elevated*			
Sample ID	*pH*	*V (g)*	*t (min)*	*Water Content (mL)*
1	5.88	85.73	60480	NS
7	5.51	122.58		NS
13	5.07	117.08		NS
Week 6	*Abiotic Ambient*			*Water Content (mL)*
Sample ID	*pH*	*V (g)*	*t (min)*	
19	NS	NS		NS
24	5.06	85.14		NS
35	5.14	100.69		NS
Week 6	*Biotic Elevated*			
Sample ID	*pH*	*V (g)*	*t (min)*	*water content (ml)*
2	4.32	55.75		10.05
3	6.59	90.11		NS
8	4.06	103.99		9.73
9	4.05	18.50		NS
10	NS	NS		NS
11	NS	NS		NS
12	NS	NS		NS
14	NS	NS		NS
15	NS	NS		NS
16	4.81	168.17		NS
17	4.88	165.96		7.57
18				
Week 6	*Biotic Ambient*			
Sample ID	*pH*	*V (g)*	*t (min)*	*water content (ml)*
21	NS	NS		NS
22	NS	NS		NS
23	NS	NS		NS
25	5.18	46.39		5.47
26	5.11	56.27		NS
27	NS	NS		NS
28	NS	NS		NS
29	NS	NS		NS
30	NS	NS		NS
31	4.77	46.78		NS
32	5.15	39.06		13.22
33	NS	NS		NS
34	NS	NS		NS

Table A-7. Week 7 pH, volume (g) and time (min).

None Detected (ND) Not Sampled (NS) Volume (V) Time (t)

Week 7	*Abiotic Elevated*		
Sample ID	*pH*	*V (g)*	*t (min)*
1	5.57	128.60	70620
7	4.76	102.83	
13	4.83	111.79	
Week 7	*Abiotic Ambient*		
Sample ID	*pH*	*V (g)*	*t (min)*
19	NS	NS	
24	4.96	110.39	
35	5.71	81.54	
Week 7	*Biotic Elevated*		
Sample ID	*pH*	*V (g)*	*t (min)*
2	4.07	63.85	
8	NS	NS	
9	4.01	57.82	
10	NS	NS	
11	NS	NS	
12	NS	NS	
14	NS	NS	
15	NS	NS	
16	4.74	161.11	
17	NS	NS	
18	NS	NS	
Week 7	*Biotic Ambient*		
Sample ID	*pH*	*V (g)*	*t (min)*
21	NS	NS	
22	NS	NS	
23	NS	NS	
25	NS	NS	
26	6.30	93.50	
27	NS	NS	
28	NS	NS	
29	NS	NS	
30	NS	NS	
31	NS	NS	
32	5.76	37.32	
33	NS	NS	
34	NS	NS	

Table A-8. Week 8 pH, volume (g), time (min) and water content of harvested plant (g)

None Detected (ND) Not Sampled (NS) Volume (V) Time (t)

Week 8 Sample ID	*Abiotic Elevated pH*	*V (g)*	*t (min)*	*Water Content (mL)*
1	5.54	113.05	79620	7.54
7	4.70	130.40		7.36
13	6.22	135.96		NS
Week 8 Sample ID	*Abiotic Ambient pH*	*V (g)*	*t (min)*	*Water Content (mL)*
19	NS	NS		NS
24	5.01	151.69		9.13
35	5.19	141.63		11.25
Week 8 Sample ID	*Biotic Elevated pH*	*V (g)*	*t (min)*	*Water Content (mL)*
2	3.77	50.28		9.8
6	NS	NS		NS
8	NS	NS		NS
9	3.92	45.58		12.93
10	NS	NS		NS
11	NS	NS		NS
12	NS	NS		NS
14	NS	NS		NS
15	NS	NS		NS
16	4.44	127.22		9.1
17	NS	NS		NS
18	NS	NS		NS
Week 8 Sample ID	*Biotic Ambient pH*	*V (g)*	*t (min)*	*Water Content (mL)*
21	NS	NS		NS
22	NS	NS		NS
23	NS	NS		NS
25	NS	NS		NS
26	4.64	116.06		8.78
27	NS	NS		NS
28	NS	NS		NS
29	NS	NS		NS
30	NS	NS		NS
31	NS	NS		NS
32	5.14	120.48		11.31
33	NS	NS		NS
34	NS	NS		NS

Appendix B Plant tissue by week Elevated CO_2 (el) Ambient CO_2 (am)

Table B-1. Week 2 plant data

Elevated CO_2 (el) Ambient CO_2 (am)

Condition	*ID*	*Dry wt(g)*	*Crucibal (g)*	*Wt Added (g)*	*Final Volume (mL)*	*Total plant (g)*	*Root: shoot*
el	*6 sh*	0.3713	10.3906	0.0576	50.35	0.4387	0.1815
el	*6 rt*	0.0674	11.1161	0.0446	50.16		
el	*11 sh*	0.2283	11.7433	0.0512	50.75	0.282	0.2352
el	*11 rt*	0.0537	11.0012	0.0499	50.08		
el	*14 sh*	0.3726	10.5891	0.0637	50.44	0.422	0.1326
el	*14 rt*	0.0494	11.6826	0.0484	50.57		
am	*21 sh*	0.2931	11.5247	0.0576	50.29	0.3463	0.1815
am	*21 rt*	0.0532	12.1897	0.0483	50.19		
am	*28 sh*	0.2821	11.6201	0.0542	50.79	0.3299	0.1694
am	*28 rt*	0.0478	10.5152	0.0451	50.03		
am	*33 sh*	0.2476	11.2049	0.0489	50.09	0.2847	0.1498
am	*33 rt*	0.0371	11.2879	0.0317	50.11		

Table B-2. Week 4 plant data

Elevated CO_2 (el) Ambient CO_2 (am)

Condition	*ID*	*Dry wt(g)*	*Crucibal (g)*	*Wt Added (g)*	*Final Volume (mL)*	*Total plant (g)*	*Root: shoot*
el	*5 sh*	0.1813	11.2915	0.0643	50.43	0.242	0.3348
el	*5 rt*	0.0607	11.6831	0.0193	50		
el	*12 sh*	0.3262	11.7432	0.0629	50.76	0.3872	0.1870
el	*12 rt*	0.0610	11.5235	0.0367	50.23		
el	*18 sh*	0.2709	10.3899	0.0555	51.46	0.3312	0.2226
el	*18 rt*	0.0603	11.0023	0.0350	50.53		
am	*22 sh*	0.2381	12.1888	0.0455	50.35	0.2915	0.2243
am	*22 rt*	0.0534	10.5167	0.0399	50.22		
am	*29 sh*	0.6286	11.1151	0.0473	50.25	0.7492	0.1919
am	*29 rt*	0.1206	11.6204	0.0610	50.71		
am	*34 sh*	0.3684	10.5880	0.0460	50.35	0.3896	0.0575
am	*34 rt*	0.0212	11.2055	0.0146	50.52		

Table B-3. Week 6 plant data

Elevated CO_2 (el) Ambient CO_2 (am)

Condition	*ID*	*Dry wt(g)*	*Crucibal (g)*	*Wt Added (g)*	*Final Volume (mL)*	*Total plant (g)*	*Root: shoot*
el	*3 sh*	0.2201	11.7432	0.0510	50.09	0.2599	0.1808
el	*3 rt*	0.0398	11.0018	0.0363	39.84		
el	*8 sh*	1.1199	11.2044	0.0548	50.12	1.3388	0.1955
el	*8 rt*	0.2189	11.6826	0.0535	50.28		
el	*17 sh*	0.1867	10.5163	0.0470	50.08	0.2259	0.2100
el	*17 rt*	0.0392	11.1157	0.0370	51.23		
am	*25 sh*	0.9513	11.2883	0.0402	49.11	1.1271	0.1848
am	*25 rt*	0.1758	11.3908	0.0643	50.2		
am	*31sh*	0.3668	12.1894	0.0566	50.45	0.4249	0.1584
am	*31 rt*	0.0581	11.6209	0.0464	39.78		

Table B-4. week 8 plant data

Elevated CO_2 (el) Ambient CO_2 (am)

Condition	*ID*	*Dry wt(g)*	*Crucibal (g)*	*Wt Added (g)*	*Final Volume (mL)*	*Total plant (g)*	*Root: shoot*
el	*2 sh*	0.9313	12.1875	0.0585	50.08	1.1716	0.2580
el	*2 rt*	0.2403	11.5231	0.0566	50.49		
el	*9 sh*	2.8001	10.3912	0.0509	50.24	3.3015	0.1791
el	*9 rt*	0.5014	11.0009	0.0533	50.55		
el	*16 sh*	0.2586	10.5166	0.0481	49.67	0.3077	0.1899
el	*16 rt*	0.0491	10.5882	0.0403	51.04		
am	*26 sh*	1.2331	11.1159	0.0586	50.03	1.3279	0.0769
am	*26 rt*	0.0948	11.6823	0.0414	49.6		
am	*32 sh*	1.4506	11.7429	0.0526	50.05	1.5726	0.0841
am	*32 rt*	0.1220	11.2882	0.0462	50.38		

Appendix C Calcium concentrations measured using AAS.

Concentrations converted to total Ca using volume (V) from weekly flow data.

Table C-1. Week 1 Ca content of leachate in mg and moles

Biotic Elevated

Not sampled (NS) None detected (ND) Harvested (H)

ID	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
2	0.045	6.6170	101.68	0.6728	1.68E-05
3	0.035	5.1846	114.28	0.5925	1.48E-05
5	0.04	5.9008	128.65	0.7591	1.89E-05
6	0.042	6.1873	118.19	0.7313	1.82E-05
8	0.049	7.1899	92.3	0.6636	1.66E-05
9	0.049	7.1899	97.98	0.7045	1.76E-05
11	0.03	4.4684	104.3	0.4661	1.16E-05
12	0.041	6.0440	95.32	0.5761	1.44E-05
14	0.101	14.6383	88.55	1.2962	3.23E-05
15	0.241	34.6915	89.77	3.1143	7.77E-05
16	0.096	13.9221	114.01	1.5873	3.96E-05
17	NS	NS	NS	NS	NS
18	0.134	19.3651	113.59	2.1997	5.49E-05
Biotic Ambient	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
21	0.089	12.9194	124.9	1.6136	4.03E-05
22	0.101	14.6383	98.83	1.4467	3.61E-05
25	0.109	15.7842	34.05	0.5375	1.34E-05
26	0.068	9.9115	47.7	0.4728	1.18E-05
27	NS	NS	NS	NS	NS
28	0.054	7.9061	40.73	0.3220	8.03E-06
29	0.157	22.6596	29.21	0.6619	1.65E-05
30	NS	NS	NS	NS	NS
31	0.036	5.3279	109.02	0.5808	1.45E-05
32	0.059	8.6223	111.54	0.9617	2.40E-05
33	0.028	4.1820	106.62	0.4459	1.11E-05
34	0.057	8.3358	97.26	0.8107	2.02E-05
Abiotic	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
1	0.05	7.3332	45.91	0.3367	8.40E-06
7	0.079	11.4871	35.69	0.4100	1.02E-05
13	0.077	11.2006	34.29	0.3841	9.58E-06
19	0.146	21.0840	117.85	2.4847	6.20E-05
24	0.117	16.9301	12.61	0.2135	5.33E-06
35	0.078	11.3438	56.93	0.6458	1.61E-05

Table C-2. Week 2 Ca content of leachate in mg and moles

Biotic Elevated

Not sampled (NS) None detected (ND) Harvested (H)

ID	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
2	0.026	3.8955	97.75	0.3808	9.50E-06
3	0.034	5.0414	87.38	0.4405	1.10E-05
4	NS	NS	NS	NS	NS
5	0.023	3.4658	64.71	0.2243	5.60E-06
6	0.046	6.7602	56.32	0.3807	9.50E-06
8	0.017	2.6063	86.4	0.2252	5.62E-06
9	0.04	5.9008	59.59	0.3516	8.77E-06
10	NS	NS	NS	NS	NS
11	0.053	7.7629	53.49	0.4152	1.04E-05
12	0.03	4.4684	84.7	0.3785	9.44E-06
14	0.014	2.1766	68.69	0.1495	3.73E-06
15	0.013	2.0334	79.73	0.1621	4.05E-06
16	0.013	2.0334	83.79	0.1704	4.25E-06
17	0.01	1.6037	59.34	0.0952	2.37E-06
18	0.023	3.4658	71.46	0.2477	6.18E-06
Biotic Ambient	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
20	NS	NS	NS	NS	NS
21	0.008	1.3172	92.01	0.1212	3.02E-06
22	0.011	1.7469	76.98	0.1345	3.36E-06
25	0.028	4.1820	106.04	0.4435	1.11E-05
26	0.025	3.7522	99.9	0.3748	9.35E-06
27	NS	NS			
28	0.017	2.6063	116.75	0.3043	7.59E-06
29	0.031	4.6117	81.51	0.3759	9.38E-06
31	0.024	4.6117	85.3	0.3934	9.82E-06
32	0.031	2.6063	89.54	0.2334	5.82E-06
33	0.017	5.4711	74.15	0.4057	1.01E-05
34	0.037	5.4711	80.2	0.4388	1.09E-05
Abiotic	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
1	0.039	5.7576	40.89	0.2354	5.87E-06
7	0.031	4.6117	29.54	0.1362	3.40E-06
13	0.051	7.4764	13.37	0.1000	2.49E-06
19	0.017	2.6063	89.6	0.2335	5.83E-06
24	0.028	4.1820	42.15	0.1763	4.40E-06
35	0.033	2.6063	58.67	0.1529	3.82E-06

Table C-3. Week 2 Ca content of plant tissue. Total Ca comes from concentration of digested plant ~50 mg times total mass of plant.

Week 2						
Elevated	mg/L (ppm)	Digested (g)	Dry wt (g)	Vol plant (ml)	Ca (mg)	Total Ca (mg)
6 sh	1.3172	0.0576	0.3713	50.35	0.0663	0.4275
6 rt	5.0414	0.0446	0.0674	50.16	0.2529	0.3821
11 sh	0.7443	0.0512	0.2283	50.75	0.0378	0.1684
11 rt	2.6063	0.0499	0.0537	50.08	0.1305	0.1405
14 sh	0.6010	0.0637	0.3726	50.44	0.0303	0.1773
14 rt	1.8902	0.0484	0.0494	50.57	0.0956	0.0976
Ambient	mg/L (ppm)	Digested (g))	Dry wt (g)	Vol plant (ml)	Ca (mg)	Total Ca (mg)
21 sh	0.6010	0.0576	0.2931	50.29	0.0302	0.1538
21 rt	0.7443	0.0483	0.0532	50.19	0.0374	0.0411
28 sh	1.1740	0.0542	0.2821	50.79	0.0596	0.3103
28 rt	0.8875	0.0451	0.0478	50.03	0.0444	0.0471
33 sh	1.1740	0.0489	0.2476	50.09	0.0588	0.2977
33 rt	4.7549	0.0317	0.0371	50.11	0.2383	0.2789

Table C-4. Week 3 Ca content of leachate in mg and moles

Biotic Elevated

Not sampled (NS) None detected (ND) Harvested (H)

ID	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
2	0.033	4.8981	151.29	0.7410	1.85E-05
3	0.04	5.9008	126.63	0.7472	1.86E-05
4	NS	NS	NS	NS	NS
5	0.034	5.0414	96.77	0.4879	1.22E-05
6	H	H	H	H	H
8	0.06	8.7656	92.71	0.8127	2.03E-05
9	0.209	30.1079	36.72	1.1056	2.76E-05
10	NS	NS	NS	NS	NS
11	H	H	H	H	H
12	0.016	2.4631	113.41	0.2793	6.97E-06
14	H	H	H	H	H
15	0.006	1.0307	183.85	0.1895	4.73E-06
16	0.006	1.0307	203.35	0.2096	5.23E-06
17	0.009	1.4604	207.8	0.3035	7.57E-06
18	0.013	2.0334	169.23	0.3441	8.59E-06
Biotic Ambient	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
20	NS	NS	NS	NS	NS
21	H	H	H	H	H
22	0.029	4.3252	83.68	0.3619	9.03E-06
23	NS	NS	NS	NS	NS
25	0.077	11.2006	115.32	1.2917	3.22E-05
26	0.047	6.9035	107.04	0.7389	1.84E-05
27	NS	NS	NS	NS	NS
28	H	H	H	H	H
29	0.074	10.7709	123.22	1.3272	3.31E-05
30	NS	NS	NS	NS	NS
31	0.041	6.0440	110.61	0.6685	1.67E-05
32	0.128	18.5057	66.39	1.2286	3.07E-05
34	0.03	4.4684	32.08	0.1433	3.58E-06
Abiotic	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
1	0.017	2.6063	110.24	0.2873	7.17E-06
7	0.011	1.7469	149.98	0.2620	6.54E-06
13	0.011	1.7469	172.33	0.3010	7.51E-06
19	0.012	1.8902	117.46	0.2220	5.54E-06
24	0.011	1.7469	174.16	0.3042	7.59E-06
35	0.013	2.0334	153.01	0.3111	7.76E-06

Table C-5. Week 4 Ca content of leachate in mg and moles

Biotic Elevated

Not sampled (NS) None detected (ND) Harvested (H)

ID	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
2	0.033	4.8981	109.03	0.5340	1.33E-05
3	0.021	3.1793	101.26	0.3219	8.03E-06
4	NS				
5	0.024	3.6090	92.31	0.3331	8.31E-06
6	H				
8	0.095	13.7789	27.94	0.3850	9.61E-06
9	0.332	47.7261	13.28	0.6338	1.58E-05
10	NS				
11	H				
12	0.022	3.3225	89.08	0.2960	7.38E-06
14	H				
15	0.006	1.0307	120.7	0.1244	3.10E-06
16	0.008	1.3172	113.23	0.1491	3.72E-06
17	0.011	1.7469	117.91	0.2060	5.14E-06
18	0.007	1.1740	104.11	0.1222	3.05E-06
Biotic Ambient	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
20	NS				
21	H				
22	0.012	1.8902	62.1	0.1174	2.93E-06
23	NS				
25	0.093	13.4924	54.76	0.7388	1.84E-05
26	0.086	12.4897	72.77	0.9089	2.27E-05
27	NS				
28	H				
29	0.072	10.4844	112.64	1.1810	2.95E-05
30	NS				
31	0.028	4.1820	76.96	0.3218	8.03E-06
32	0.177	25.5243	31.83	0.8124	2.03E-05
34	0.025	3.7522	106.83	0.4009	1.00E-05
Abiotic	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
1	0.013	2.0334	107.13	0.2178	5.44E-06
7	0.008	1.3172	117.26	0.1545	3.85E-06
13	0.01	1.6037	121.23	0.1944	4.85E-06
19	0.009	1.4604	86.81	0.1268	3.16E-06
24	0.008	1.3172	126.05	0.1660	4.14E-06
35	0.008	1.3172	113.56	0.1496	3.73E-06

Table C-6. Week 4 Ca content of plant tissue. Total Ca comes from concentration of digested plant ~50 mg times total mass of plant.

Elevated	mg/L (ppm)	Digested (g)	Dry wt (g)	Vol plant (ml)	Ca (mg)	Total Ca (mg)
5 sh	1.0307	0.0643	0.1813	50.43	0.0520	0.1466
5 rt	2.1766	0.0193	0.0607	50	0.1088	0.3423
12 sh	1.0307	0.0629	0.3262	50.76	0.0523	0.2713
12 rt	3.1793	0.0367	0.061	50.23	0.1597	0.2654
18 sh	1.1740	0.0555	0.2709	51.46	0.0604	0.2949
18 rt	2.3199	0.0350	0.0603	50.53	0.1172	0.2020
Ambient	mg/L (ppm)	Digested (g)	Dry wt (g)	Vol plant (ml)	Ca (mg)	Total Ca (mg)
22 sh	1.1740	0.0455	0.2381	50.35	0.0591	0.3093
22 rt	5.6143	0.0399	0.0534	50.22	0.2820	0.3773
29 sh	0.8875	0.0473	0.6286	50.25	0.0446	0.5927
29 rt	4.1820	0.0610	0.1206	50.71	0.2121	0.4193
34 sh	0.8875	0.0460	0.3684	50.35	0.0447	0.3579
34 rt	3.1793	0.0146	0.0212	50.52	0.1606	0.2332

Table C-7. Week 5 Ca content of leachate in mg and moles

Biotic Elevated

Not sampled (NS) None detected (ND) Harvested (H)

ID	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
2	0.031	4.6117	100.73	0.4645	1.16E-05
3	0.012	1.8902	88.07	0.1665	4.15E-06
4	NS				
5	H				
6	H				
8	0.049	7.1899	135.64	0.9752	2.43E-05
9	0.367	52.7395	20.67	1.0901	2.72E-05
10	NS				
11	H				
12	H				
14	H				
15	NS				
16	0.01	1.6037	100.68	0.1615	4.03E-06
17	0.009	1.4604	108.45	0.1584	3.95E-06
18	H				
Biotic Ambient	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
20	NS				
21	H				
22	H				
23	NS				
25	0.048	7.0467	136.36	0.9609	2.40E-05
26	0.044	6.4738	152.24	0.9856	2.46E-05
27	NS				
28	H				
29	H				
30	NS				
31	0.054	7.9061	68.04	0.5379	1.34E-05
32	0.058	8.4791	133.94	1.1357	2.83E-05
33	H				
34	H				
Abiotic	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
1	0.011	1.7469	109.55	0.1914	4.78E-06
7	0.006	1.0307	142.84	0.1472	3.67E-06
13	0.018	2.7496	109.9	0.3022	7.54E-06
19	NS				
24	0.007	1.1740	108.81	0.1277	3.19E-06
35	0.006	0.5940	128.03	0.0761	1.90E-06

Table C-8. Week 6 Ca content of leachate in mg and moles

Biotic Elevated

Not sampled (NS) None detected (ND) Harvested (H)

ID	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
2	0.085	11.6963	55.75	0.6521	1.63E-05
3	0.007	0.7346	90.11	0.0662	1.65E-06
4	NS	NS	NS	NS	NS
5	H	H	H	H	H
6	H	H	H	H	H
8	0.069	9.4477	103.99	0.9825	2.45E-05
9	0.575	80.5583	18.5	1.4903	3.72E-05
10	NS	NS	NS	NS	NS
11	H	H	H	H	H
12	H	H	H	H	H
14	H	H	H	H	H
15	NS	NS	NS	NS	NS
16	0.009	1.0156	168.17	0.1708	4.26E-06
17	0.007	0.7346	165.96	0.1219	3.04E-06
18	H				

Biotic Ambient	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
20	NS	NS	NS	NS	NS
21	H	H	H	H	H
22	H	H	H	H	H
23	NS	NS	NS	NS	NS
25	0.055	7.4802	46.39	0.3470	8.66E-06
26	0.037	4.9506	56.27	0.2786	6.95E-06
27	NS	NS	NS	NS	NS
28	H	H	H	H	H
29	H	H	H	H	H
30	NS	NS	NS	NS	NS
31	0.072	9.8693	46.78	0.4617	1.15E-05
32	0.095	13.1016	39.06	0.5117	1.28E-05
33	H				
34	H				

Abiotic	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
1	0.008	0.8751	85.73	0.0750	1.87E-06
7	0.003	0.1724	122.58	0.0211	5.27E-07
13	0.007	0.7346	117.08	0.0860	2.15E-06
19	NS	NS	NS	NS	NS
24	0.006	0.5940	85.14	0.0506	1.26E-06
35	0.004	0.3130	100.69	0.0315	7.86E-07

Table C-9. Week 6 Ca content of plant tissue. Total Ca comes from concentration of digested plant ~50 mg times total mass of plant.

None detected (ND)

Elevated	mg/L (ppm)	Digested (g)	Dry wt (g)	Vol plant (ml)	Ca (mg)	Total Ca (mg)
3sh	0.8751	0.0510	0.2201	50.09	0.0438	0.1892
3 rt	2.8426	0.0363	0.0398	39.84	0.1132	0.1242
8 sh	ND	ND	ND	ND	ND	ND
8 rt	0.3130	0.0535	0.2189	50.28	0.0157	0.0644
17 sh	1.0156	0.0470	0.1867	50.08	0.0509	0.2020
17 rt	8.1829	0.0370	0.0392	51.23	0.4192	0.4441
Ambient	mg/L (ppm)	Digested (g)	Dry wt (g)	Vol plant (ml)	Ca (mg)	Total Ca (mg)
25 sh	0.4535	0.0402	0.9513	49.11	0.0223	0.5270
25 rt	1.1562	0.0643	0.1758	50.2	0.0580	0.1587
31 sh	ND	ND	ND	ND	ND	ND
31 rt	0.7346	0.0464	0.0581	39.78	0.0292	0.0366

Table C-10. Week 7 Ca content of leachate in mg and moles

Biotic Elevated

Not sampled (NS) None detected (ND) Harvested (H)

ID	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
2	0.161	22.3769	63.85	1.4288	3.56E-05
3	H	H	H	H	H
4	NS	NS	NS	NS	NS
5	H	H	H	H	H
6	H	H	H	H	H
8	NS	NS	NS	NS	NS
9	0.327	45.7057	57.82	2.6427	6.59E-05
10	NS	NS	NS	NS	NS
11	H	H	H	H	H
12	H	H	H	H	H
14	H	H	H	H	H
15	NS	NS	NS	NS	NS
16	0.011	1.2967	161.11	0.2089	5.21E-06
17	H	H	H	H	H
18	H	H	H	H	H

Biotic Ambient	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
20	NS	NS	NS	NS	NS
21	H	H	H	H	H
22	H	H	H	H	H
23	NS	NS	NS	NS	NS
25	H	H	H	H	H
26	0.0894	12.3146	93.5	1.1514	2.87E-05
27	NS	NS	NS	NS	NS
28	H	H	H	H	H
29	H	H	H	H	H
30	NS	NS	NS	NS	NS
31	H	H	H	H	H
32	0.103	14.2259	37.32	0.5309	1.32E-05
33	H	H	H	H	H
34	H	H	H	H	H

Abiotic	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
1	0.005	0.4535	128.6	0.0583	1.46E-06
7	0.014	1.7183	102.83	0.1767	4.41E-06
13	0.01	1.1562	111.79	0.1292	3.22E-06
19	NS	NS	NS	NS	NS
24	0.002	0.0319	110.39	0.0035	8.78E-08
35	0.005	0.4535	81.54	0.0370	9.23E-07

Table C-11. Week 8 Ca content of leachate in mg and moles

Biotic Elevated

Not sampled (NS) None detected (ND) Harvested (H)

ID	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
2	0.224	31.2306	50.28	1.5703	3.92E-05
3	H	H	H	H	H
4	NS	NS	NS	NS	NS
5	H	H	H	H	H
6	H	H	H	H	H
8	NS	NS	NS	NS	NS
9	0.339	47.3921	45.58	2.1601	5.39E-05
10	NS	NS	NS	NS	NS
11	H	H	H	H	H
12	H	H	H	H	H
14	H	H	H	H	H
15	NS	NS	NS	NS	NS
16	0.023	2.9831	127.22	0.3795	9.47E-06
17	H	H	H	H	H
18	H	H	H	H	H
Biotic Ambient	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
20	NS	NS	NS	NS	NS
21	H	H	H	H	H
22	H	H	H	H	H
23	NS	NS	NS	NS	NS
25	H	H	H	H	H
26	0.041	5.5127	116.06	0.6398	1.60E-05
27	NS				
28	H	H	H	H	H
29	H	H	H	H	H
30	NS	NS	NS	NS	NS
31	H	H	H	H	H
32	0.023	2.9831	120.48	0.3594	8.97E-06
33	H	H	H	H	H
34	H	H	H	H	H
Abiotic	Absorbance	mg/L (ppm)	V (mL)	Ca (mg)	m $_{Ca}$
1	0.005	0.4535	113.05	0.0513	1.28E-06
7	0.009	1.0156	130.4	0.1324	3.30E-06
13	0.006	0.5940	135.96	0.0808	2.02E-06
19	NS	NS	NS	NS	NS
24	0.002	0.0319	151.69	0.0048	1.21E-07
35	0.001	ND	141.63	ND	ND

Table C-12. Week 8 Ca content of plant tissue. Total Ca comes from concentration of digested plant ~50 mg times total mass of plant.

None detected (ND)

Elevated	mg/L (ppm)	Digested (g)	Dry wt (g)	Vol plant (ml)	Ca (mg)	Total Ca (mg)
2 sh	0.3130	0.0585	0.9313	50.08	0.0157	0.2495
2 rt	1.7183	0.0566	0.2403	50.49	0.0868	0.3683
9 sh	0.4535	0.0509	2.8001	50.24	0.0228	1.2534
9 rt	3.9669	0.0533	0.5014	50.55	0.2005	1.8864
16 sh	1.0156	0.0481	0.2586	49.67	0.0504	0.2712
16 rt	13.1016	0.0403	0.0491	51.04	0.6687	0.8147
Ambient	mg/L (ppm)	Digested (g)	Dry wt (g)	Vol plant (ml)	Ca (mg)	Total ca (mg)
26 sh	0.7346	0.0586	1.2331	50.03	0.0368	0.7733
26 rt	1.4372	0.0414	0.0948	49.6	0.0713	0.1632
32 sh	ND	ND	ND	ND	ND	ND
32 rt	8.7450	0.0462	0.122	50.38	0.4406	1.1634
Bean seed	0.7346	0.0544	0.43	50.16	0.0368	0.2912

Appendix D P concentrations measured using colorimetry.

Concentrations converted to total P using volume (V) from weekly flow data.

Table D-1. Week 1 P content of leachate in mg and moles.

Biotic Elevated

None Detected (ND) Not Sampled (NS) Harvested (H)

Sample ID	mg/L	Dilution	mg/L	V (ml)	P (mg)	m_P
2	0.0707	9.29	0.6567	101.68	0.0668	2.16E-06
3	0.0100	9.29	0.0931	114.28	0.0106	3.43E-07
4	NS	NS	NS	NS	NS	NS
5	0.0614	9.29	0.5708	128.65	0.0734	2.37E-06
6	0.0083	9.29	0.0771	118.19	0.0091	2.94E-07
8	ND	ND	ND	ND	ND	ND
9	0.0066	9.29	0.0615	97.98	0.0060	1.95E-07
10	NS	NS	NS	NS	NS	NS
11	0.0019	9.29	0.0176	104.30	0.0018	5.93E-08
12	0.0017	9.29	0.0154	95.32	0.0015	4.75E-08
14	ND	ND	ND	ND	ND	ND
15	ND	ND	ND	ND	ND	ND
16	ND	ND	ND	ND	ND	ND
17	ND	ND	ND	ND	ND	ND
18	ND	ND	ND	ND	ND	ND
Biotic Ambient	mg/L	Dilution	mg/L	V (ml)	P (mg)	m_P
20	ND	ND	ND	ND	ND	ND
21	ND	ND	ND	ND	ND	ND
22	ND	ND	ND	ND	ND	ND
23	ND	ND	ND	ND	ND	ND
25	0.0991	9.29	0.9210	34.05	0.0314	1.01E-06
26	0.0394	9.29	0.3665	47.70	0.0175	5.64E-07
28	0.0005	9.29	0.0050	40.73	0.0002	6.53E-09
29	0.1697	9.29	1.5763	29.21	0.0460	1.49E-06
31	0.0043	9.29	0.0403	109.02	0.0044	1.42E-07
32	0.0025	9.29	0.0229	111.54	0.0025	8.23E-08
33	0.0016	9.29	0.0146	106.62	0.0016	5.01E-08
34	0.0015	9.29	0.0138	97.26	0.0013	4.35E-08
Abiotic	mg/L	Dilution	mg/L	V (ml)	P (mg)	m_P
1	0.0025	4.15	0.0105	45.91	0.0005	1.56E-08
7	0.0051	4.15	0.0211	35.69	0.0008	2.43E-08
13	0.0255	4.15	0.1056	34.29	0.0036	1.17E-07
24	0.0280	9.29	0.2603	12.61	0.0033	1.06E-07
35	0.0166	4.15	0.0690	56.93	0.0039	1.27E-07

Table D-2. Week 2 P content of leachate in mg and moles.

Biotic Elevated

None Detected (ND) Not Sampled (NS) Harvested (H)

Sample ID	*mg/L*	*Dilution*	*mg/L*	*mL*	*P (mg)*	*m P*
2	0.0754	9.29	0.7000	97.75	0.0684	2.21E-06
3	0.0309	9.29	0.2871	87.38	0.0251	8.10E-07
4	NS	NS	NS	NS	NS	NS
5	0.0189	9.29	0.1760	64.71	0.0114	3.68E-07
6	0.0420	9.29	0.3900	56.32	0.0220	7.09E-07
8	0.0038	9.29	0.0354	86.40	0.0031	9.86E-08
9	0.0079	9.29	0.0732	59.59	0.0044	1.41E-07
10	NS	NS	NS	NS	NS	NS
11	0.0486	9.29	0.4520	53.49	0.0242	7.80E-07
12	0.0023	9.29	0.0211	84.70	0.0018	5.77E-08
14	0.0152	9.29	0.1414	68.69	0.0097	3.13E-07
15	0.0252	9.29	0.2341	79.73	0.0187	6.03E-07
16	0.0158	9.29	0.1470	83.79	0.0123	3.98E-07
17	0.0238	9.29	0.2215	59.34	0.0131	4.24E-07
18	0.0232	9.29	0.2160	71.46	0.0154	4.98E-07
Biotic Ambient	*mg/L*	*Dilution*	*mg/L*	*mL*	*P (mg)*	*m P*
20						
21	0.0177	9.29	0.1643	92.01	0.0151	4.88E-07
22	NS	NS	NS	NS	NS	NS
23	NS	NS	NS	NS	NS	NS
25	0.0112	9.29	0.1041	106.04	0.0110	3.57E-07
26	0.0311	9.29	0.2885	99.90	0.0288	9.31E-07
27	NS	NS	NS	NS	NS	NS
28	0.0014	9.29	0.0127	116.75	0.0015	4.78E-08
29	NS	NS	NS	NS	NS	NS
30	NS	NS	NS	NS	NS	NS
31	0.0007	9.29	0.0063	85.30	0.0005	1.73E-08
32	0.0009	9.29	0.0085	89.54	0.0008	2.45E-08
33	0.0022	9.29	0.0205	74.15	0.0015	4.91E-08
34	0.0010	9.29	0.0092	80.20	0.0007	2.38E-08
Abiotic	*mg/L*	*Dilution*	*mg/L*	*mL*	*P (mg)*	*m P*
1	0.0395	4.145	0.1637	40.89	0.0067	2.16E-07
7	0.0106	4.145	0.0437	29.54	0.0013	4.17E-08
13	0.0013	9.29	0.0124	13.37	0.0002	5.35E-09
19	0.0585	4.145	0.2426	89.60	0.0217	7.02E-07
24	0.0307	4.145	0.1273	42.15	0.0054	1.73E-07
35	0.0034	4.145	0.0143	58.67	0.0008	2.71E-08

Table D-3. Week 2 P content of plant tissue. Total P comes from concentration of digested plant ~50 mg times total mass of plant.

Elevated	mg/l	Dilution	mg/l	Vol plant (ml)	Digested (g)	Dry wt (g)	Total P (mg)
6 sh	0.0715	0.0086	8.2974	50.35	0.0576	0.3713	2.6930
6 rt	0.0921	0.0086	10.6825	50.16	0.0446	0.0674	0.8098
11 sh	0.0736	0.0086	8.5366	50.75	0.0512	0.2283	1.9318
11 rt	0.0638	0.0086	7.3970	50.08	0.0499	0.0537	0.3987
14 sh	0.0873	0.0086	10.1306	50.44	0.0637	0.3726	2.9889
14 rt	0.0632	0.0086	7.3303	50.57	0.0484	0.0494	0.3784
Elevated	mg/l	Dilution	mg/l	Vol plant	Digested (g)	Dry wt (g)	Total P (mg)
21 sh	0.0782	0.0086	9.0679	50.29	0.0576	0.2931	2.3205
21 rt	0.0638	0.0086	7.4038	50.19	0.0483	0.0532	0.4093
28 sh	0.0579	0.0086	6.7136	50.79	0.0542	0.2821	1.7747
28 rt	0.0540	0.0086	6.2659	50.03	0.0451	0.0478	0.3323
33 sh	0.0723	0.0086	8.3879	50.09	0.0489	0.2476	2.1274
33 rt	0.0703	0.0086	8.1504	50.11	0.0317	0.0371	0.4780

Table D-4. Week 3 P content of leachate in mg and moles.

Biotic Elevated

None Detected (ND) Not Sampled (NS) Harvested (H)

Sample ID	*mg/L*	*Dilution*	*mg/L*	*mL*	*P (mg)*	m_P
2	0.1268	9.29	1.1781	151.29	0.1782	5.75E-06
3	0.0742	9.29	0.6890	126.63	0.0872	2.82E-06
4	NS	NS	NS	NS	NS	NS
5	0.0428	9.29	0.3976	96.77	0.0385	1.24E-06
6	H	H	H	H	H	H
8	0.0427	9.29	0.3964	92.71	0.0368	1.19E-06
9	0.0327	9.29	0.3039	36.72	0.0112	3.60E-07
10	0.0014	9.29	0.0128	164.68	0.0021	6.78E-08
11	H	H	H	H	H	H
12	0.0049	9.29	0.0455	113.41	0.0052	1.67E-07
14	H	H	H	H	H	H
15	0.0128	9.29	0.1192	183.85	0.0219	7.08E-07
16	0.0103	9.29	0.0959	203.35	0.0195	6.30E-07
17	0.0268	9.29	0.2494	207.80	0.0518	1.67E-06
18	0.0354	9.29	0.3292	169.23	0.0557	1.80E-06
Biotic Ambient	*mg/L*	*Dilution*	*mg/L*	*mL*	*P (mg)*	m_P
20	NS	NS	NS	NS	NS	NS
21	H	H	H	H	H	H
22	0.0207	9.29	0.1923	83.68	0.0161	5.20E-07
23	NS	NS	NS	NS	NS	NS
25	0.1461	9.29	1.3571	115.32	0.1565	5.05E-06
26	0.0826	9.29	0.7678	107.04	0.0822	2.65E-06
27	NS	NS	NS	NS	NS	NS
28	H	H	H	H	H	H
29	0.0739	9.29	0.6866	123.22	0.0846	2.73E-06
30	NS	NS	NS	NS	NS	NS
31	0.0224	9.29	0.2080	110.61	0.0230	7.43E-07
32	0.0125	9.29	0.1164	66.39	0.0077	2.50E-07
33	H	H	H	H	H	H
34	0.0067	9.29	0.0623	32.08	0.0020	6.45E-08
Abiotic	*mg/L*	*Dilution*	*mg/L*	*mL*	*P (mg)*	m_P
1	0.0850	4.145	0.3525	110.24	0.0389	1.25E-06
7	0.0118	4.145	0.0490	149.98	0.0074	2.37E-07
13	0.0107	4.145	0.0445	172.33	0.0077	2.48E-07
19	0.0079	4.145	0.0328	117.46	0.0038	1.24E-07
24	0.0104	4.145	0.0430	174.16	0.0075	2.42E-07
35	0.0047	4.145	0.0197	153.01	0.0030	9.72E-08

Table D-5. Week 4 P content of leachate in mg and moles.

Biotic Elevated

None Detected (ND) Not Sampled (NS) Harvested (H)

Sample ID	*mg/L*	*Dilution*	*mg/L*	*mL*	*P (mg)*	*m P*
2	0.0984	9.29	0.9145	109.03	0.0997	3.22E-06
3	0.0364	9.29	0.3381	101.26	0.0342	1.11E-06
4	NS	NS	NS	NS	NS	NS
5	0.0273	9.29	0.2535	92.31	0.0234	7.55E-07
6	H	H	H	H	H	H
8	0.0317	9.29	0.2943	27.94	0.0082	2.65E-07
9	0.0541	9.29	0.5026	13.28	0.0067	2.15E-07
10	NS	NS	NS	NS	NS	NS
11	H	H	H	H	H	H
12	0.0034	9.29	0.0312	89.08	0.0028	8.98E-08
14	H	H	H	H	H	H
15	0.0075	9.29	0.0697	120.70	0.0084	2.72E-07
16	0.0061	9.29	0.0565	113.23	0.0064	2.07E-07
17	0.0210	9.29	0.1946	117.91	0.0230	7.41E-07
18	0.0195	9.29	0.1811	104.11	0.0189	6.09E-07
Biotic Ambient	*mg/L*	*Dilution*	*mg/L*	*mL*	*P (mg)*	*m P*
20	NS	NS	NS	NS	NS	NS
21	H	H	H	H	H	H
22	0.0144	9.29	0.1338	62.10	0.0083	2.68E-07
23	NS	NS	NS	NS	NS	NS
25	0.0804	9.29	0.7472	54.76	0.0409	1.32E-06
26	0.0813	9.29	0.7554	72.77	0.0550	1.77E-06
27	NS	NS	NS	NS	NS	NS
28	H	H	H	H	H	H
29	0.1028	9.29	0.9551	112.64	0.1076	3.47E-06
30	NS	NS	NS	NS	NS	NS
31	NS	NS	NS	NS	NS	NS
32	1.3044	9.29	12.1182	31.83	0.3857	1.25E-05
33	H	H	H	H	H	H
34	0.0080	9.29	0.0742	106.83	0.0079	2.56E-07
Abiotic	*mg/L*	*Dilution*	*mg/L*	*mL*	*P (mg)*	*m P*
1	0.0476	4.145	0.1975	107.13	0.0212	6.83E-07
7	0.0076	4.145	0.0313	117.26	0.0037	1.19E-07
13	0.0071	4.145	0.0295	121.23	0.0036	1.16E-07
19	0.0093	4.145	0.0387	86.81	0.0034	1.08E-07
24	0.0114	4.145	0.0471	126.05	0.0059	1.92E-07
35	0.0072	4.145	0.0299	113.56	0.0034	1.09E-07

Table D-6. Week 4 P content of plant tissue. Total P comes from concentration of digested plant ~50 mg times total mass of plant.

Elevated	mg/l	Dilution	mg/l	Vol plant (ml)	Digested (g)	Dry wt (g)	Total P (mg)
5 sh	0.0863	0.0086	10.0125	50.43	0.0643	0.1813	1.4237
5 rt	0.0266	0.0086	3.0852	50.00	0.0193	0.0607	0.4852
12 sh	0.0832	0.0086	9.6547	50.76	0.0629	0.3262	2.5415
12 rt	0.0545	0.0086	6.3274	50.23	0.0367	0.061	0.5283
18 sh	0.1125	0.0086	13.0516	51.46	0.0555	0.2709	3.2783
18 rt	0.0560	0.0086	6.5000	50.53	0.0350	0.0603	0.5659
Elevated	mg/l	Dilution	mg/l	Vol plant (ml)	Digested (g)	Dry wt (g)	Total P (mg)
22 sh	0.0637	0.0086	7.3936	50.35	0.0455	0.2381	1.9481
22 rt	0.0571	0.0086	6.6281	50.22	0.0399	0.0534	0.4455
29 sh	0.0326	0.0086	3.7766	50.25	0.0473	0.6286	2.5220
29 rt	0.0565	0.0086	6.5581	50.71	0.0610	0.1206	0.6575
34 sh	0.0732	0.0086	8.4887	50.35	0.0460	0.3684	3.4230
34 rt	0.0231	0.0086	2.6815	50.52	0.0146	0.0212	0.1967

Table D-7. Week 5 P content of leachate in mg and moles.

Biotic Elevated

None Detected (ND) Not Sampled (NS) Harvested (H)

Sample ID	*mg/L*	*Dilution*	*mg/L*	*mL*	*P (mg)*	*m P*
2	0.0475	9.29	0.4411	100.73	0.0444	1.43E-06
3	0.0159	9.29	0.1480	88.07	0.0130	4.21E-07
4	NS	NS	NS	NS	NS	NS
5	H	H	H	H	H	H
6	H	H	H	H	H	H
8	0.0118	9.29	0.1101	135.64	0.0149	4.82E-07
9	0.0673	9.29	0.6252	20.67	0.0129	4.17E-07
10	NS	NS	NS	NS	NS	NS
11	H	H	H	H	H	H
12	H	H	H	H	H	H
14	H	H	H	H	H	H
15	NS	NS	NS	NS	NS	NS
16	0.0035	9.29	0.0324	100.68	0.0033	1.05E-07
17	0.0045	9.29	0.0415	108.45	0.0045	1.45E-07
18	NS	NS	NS	NS	NS	NS
Biotic Ambient	*mg/L*	*Dilution*	*mg/L*	*mL*	*P (mg)*	*m P*
20	NS	NS	NS	NS	NS	NS
21	H	H	H	H	H	H
22	H	H	H	H	H	H
23	NS	NS	NS	NS	NS	NS
25	0.0340	9.29	0.3157	136.36	0.0431	1.39E-06
26	0.0129	9.29	0.1199	152.24	0.0183	5.89E-07
27	NS	NS	NS	NS	NS	NS
28	H	H	H	H	H	H
29	H	H	H	H	H	H
30	NS	NS	NS	NS	NS	NS
31	0.0326	9.29	0.3026	68.04	0.0206	6.65E-07
32	0.0119	9.29	0.1108	133.94	0.0148	4.79E-07
33	H	H	H	H	H	H
34	H	H	H	H	H	H
Abiotic	*mg/L*	*Dilution*	*mg/L*	*mL*	*P (mg)*	*m P*
1	0.0046	4.145	0.0193	109.55	0.0021	6.81E-08
7	0.0039	4.145	0.0162	142.84	0.0023	7.47E-08
13	0.0066	4.145	0.0276	109.90	0.0030	9.78E-08
19	NS	NS	NS	NS	NS	NS
24	0.0126	4.145	0.0524	108.81	0.0057	1.84E-07
35	0.0029	4.145	0.0121	128.03	0.0016	5.01E-08

Table D-8. Week 6 P content of leachate in mg and moles.

Biotic Elevated

None Detected (ND) Not Sampled (NS) Harvested (H)

Sample ID	mg/L	Dilution	mg/L	mL	P (mg)	m_P
2	0.0471	9.29	0.4378	55.75	0.0244	7.88E-07
3	0.0690	9.29	0.6414	90.11	0.0578	1.87E-06
4	NS	NS	NS	NS	NS	NS
5	H	H	H	H	H	H
6	H	H	H	H	H	H
8	0.0060	9.29	0.0556	103.99	0.0058	1.87E-07
9	0.0462	9.29	0.4289	18.5	0.0079	2.56E-07
10	NS	NS	NS	NS	NS	NS
11	H	H	H	H	H	H
12	H	H	H	H	H	H
14	H	H	H	H	H	H
15	NS	NS	NS	NS	NS	NS
16	0.0102	9.29	0.0952	168.17	0.0160	5.17E-07
17	0.0060	9.29	0.0562	165.96	0.0093	3.01E-07
18						
Biotic Ambient	mg/L	Dilution	mg/L	mL	P (mg)	m_P
20	NS	NS	NS	NS	NS	NS
21	H	H	H	H	H	H
22	H	H	H	H	H	H
23	NS	NS	NS	NS	NS	NS
25	0.0106	9.29	0.0984	46.39	0.0046	1.47E-07
26	0.0061	9.29	0.0570	56.27	0.0032	1.04E-07
27	NS	NS	NS	NS	NS	NS
28	H	H	H	H	H	H
29	H	H	H	H	H	H
30	NS	NS	NS	NS	NS	NS
31	0.0379	9.29	0.3524	46.78	0.0165	5.32E-07
32	0.0074	9.29	0.0683	39.06	0.0027	8.62E-08
33	H	H	H	H	H	H
34	H	H	H	H	H	H
Abiotic	mg/L	Dilution	mg/L	mL	P (mg)	m_P
1	0.0070	4.145	0.0291	85.73	0.0025	8.07E-08
7	0.0046	4.145	0.0190	122.58	0.0023	7.53E-08
13	0.0022	4.145	0.0091	117.08	0.0011	3.43E-08
19	NS	NS	NS	NS	NS	NS
24	0.0087	4.145	0.0362	85.14	0.0031	9.94E-08
35	0.0046	4.145	0.0190	100.69	0.0019	6.16E-08

Table D-9. Week 6 P content of plant tissue. Total P comes from concentration of digested plant ~50 mg times total mass of plant.

Elevated	mg/l	Dilution	mg/l	Vol plant (ml)	Digested (g)	Dry wt (g)	Total P (mg)
3sh	0.0777	0.0086	9.0098	50.09	0.0510	0.2201	1.9477
3 rt	0.0546	0.0086	6.3292	39.84	0.0363	0.0398	0.2765
8 sh	0.0238	0.0086	2.7584	50.12	0.0548	1.1199	2.8253
8 rt	0.0238	0.0086	2.7652	50.28	0.0535	0.2189	0.5689
17 sh	0.0664	0.0086	7.7062	50.08	0.0470	0.1867	1.5330
17 rt	0.0654	0.0086	7.5900	51.23	0.0370	0.0392	0.4120
Elevated	mg/l	Dilution	mg/l	Vol plant (ml)	Digested (g)	Dry wt (g)	Total P (mg)
25 sh	0.0244	0.0086	2.8318	49.11	0.0402	0.9513	3.2910
25 rt	0.0362	0.0086	4.1952	50.2	0.0643	0.1758	0.5758
31 sh	0.0397	0.0086	4.6018	50.45	0.0566	0.3668	1.5045
31 rt	0.0401	0.0086	4.6463	39.78	0.0464	0.0581	0.2314

Table D-10. Week 7 P content of leachate in mg and moles.

Biotic Elevated

None Detected (ND) Not Sampled (NS) Harvested (H)

Sample ID	*mg/L*	*Dilution*	*mg/L*	*mL*	*P (mg)*	*m P*
2	0.0512	9.29	0.4755	63.85	0.0304	9.80E-07
3	H	H	H	H	H	H
4	NS	NS	NS	NS	NS	NS
5	H	H	H	H	H	H
6	H	H	H	H	H	H
8	H	H	H	H	H	H
9	0.0115	9.29	0.1068	57.82	0.0062	1.99E-07
10	NS	NS	NS	NS	NS	NS
11	H	H	H	H	H	H
12	H	H	H	H	H	H
14	H	H	H	H	H	H
15	NS	NS	NS	NS	NS	NS
16	0.0045	9.29	0.0417	161.11	0.0067	2.17E-07
17	NS	NS	NS	NS	NS	NS
18	NS	NS	NS	NS	NS	NS
Biotic Ambient	*mg/L*	*Dilution*	*mg/L*	*mL*	*P (mg)*	*m P*
20	NS	NS	NS	NS	NS	NS
21	H	H	H	H	H	H
22	H	H	H	H	H	H
23	NS	NS	NS	NS	NS	NS
25	H	H	H	H	H	H
26	H	H	H	H	H	H
27	NS	NS	NS	NS	NS	NS
28	H	H	H	H	H	H
29	H	H	H	H	H	H
30	NS	NS	NS	NS	NS	NS
31	H	H	H	H	H	H
32	0.0060	9.29	0.0561	37.32	0.0021	6.75E-08
33	H	H	H	H	H	H
34	H	H	H	H	H	H
Abiotic	*mg/L*	*Dilution*	*mg/L*	*mL*	*P (mg)*	*m P*
1	0.0335	4.145	0.1387	128.6	0.0178	5.76E-07
7	0.0036	4.145	0.0148	102.83	0.0015	4.91E-08
13	0.0032	4.145	0.0135	111.79	0.0015	4.86E-08
19	NS	NS	NS	NS	NS	NS
24	0.0024	4.145	0.0101	110.39	0.0011	3.59E-08
35	0.0019	4.145	0.0080	81.54	0.0007	2.11E-08

Table D-11. Week 8 P content of leachate in mg and moles.

Biotic Elevated

None Detected (ND) Not Sampled (NS) Harvested (H)

Sample ID	mg/L	Dilution	mg/L	mL	P (mg)	m_P
2	0.0513	9.29	0.4764	50.28	0.0240	7.73E-07
3	H	H	H	H	H	H
4	NS	NS	NS	NS	NS	NS
5	H	H	H	H	H	H
6	H	H	H	H	H	H
8	H	H	H	H	H	H
9	0.0128	9.29	0.1191	45.58	0.0054	1.75E-07
10	NS	NS	NS	NS	NS	NS
11	H	H	H	H	H	H
12	H	H	H	H	H	H
14	H	H	H	H	H	H
15	NS	NS	NS	NS	NS	NS
16	0.0157	9.29	0.1459	127.22	0.0186	5.99E-07
17						
18						
Biotic Ambient	mg/L	Dilution	mg/L	mL	P (mg)	m_P
20	NS	NS	NS	NS	NS	NS
21	H	H	H	H	H	H
22	H	H	H	H	H	H
23	NS	NS	NS	NS	NS	NS
25	H	H	H	H	H	H
26	0.0149	9.29	0.1383	116.06	0.0161	5.18E-07
27	NS	NS	NS	NS	NS	NS
28	H	H	H	H	H	H
29	H	H	H	H	H	H
30	NS	NS	NS	NS	NS	NS
31	H	H	H	H	H	H
32	0.0057	9.29	0.0530	120.48	0.0064	2.06E-07
33	H	H	H	H	H	H
34	H	H	H	H	H	H
Abiotic	mg/L	Dilution	mg/L	mL	P (mg)	m_P
1	0.0028	4.145	0.0117	113.05	0.0013	4.26E-08
7	0.0032	4.145	0.0135	130.4	0.0018	5.67E-08
13	0.0405	4.145	0.1679	135.96	0.0228	7.37E-07
19	NS	NS	NS	NS	NS	NS
24	0.0033	4.145	0.0138	151.69	0.0021	6.77E-08
35	0.0024	4.145	0.0099	141.63	0.0014	4.55E-08

Table D-12. Week 8 P content of plant tissue. Total P comes from concentration of digested plant ~50 mg times total mass of plant.

Elevated	mg/l	Dilution	mg/l	Vol plant (ml)	Digested (g)	Dry wt (g)	Total P (mg)
2 sh	0.0525	0.008620	6.0952	50.08	0.0585	0.9313	4.8595
2 rt	0.0353	0.008620	4.0989	50.49	0.0566	0.2403	0.8786
9 sh	0.0264	0.008620	3.0659	50.24	0.0509	2.8001	8.4734
9 rt	0.0432	0.008620	5.0100	50.55	0.0533	0.5014	2.3824
16 sh	0.0507	0.008620	5.8805	49.67	0.0481	0.2586	1.5703
16 rt	0.1163	0.008620	13.4907	51.04	0.0403	0.0491	0.8389
Elevated	mg/l	Dilution	mg/l	Vol plant (ml)	Digested (g)	Dry wt (g)	Total P (mg)
26 sh	0.0288	0.008620	3.3367	50.03	0.0586	1.2331	3.5128
26 rt	0.0387	0.008620	4.4877	49.6	0.0414	0.0948	0.5097
32 sh	0.0262	0.008620	3.0427	50.05	0.0526	1.4506	4.1997
32 rt	0.0939	0.008620	10.8869	50.38	0.0462	0.1220	1.4484
Bean seed	0.0520	0.008620	6.0372	50.16	0.2898	0.4300	0.4493

Appendix E Total Ca released by with time.

Individual plant pots occupy separate columns.

Table E-1. Abiotic experiments

Mg Ca Leachate	Abiotic Elevated			Abiotic Ambient		
week	1	7	13	19	24	35
Week 1	0.3367	0.4100	0.3841	0.2485	0.2135	0.6458
Week 2	0.2354	0.1362	0.1000	0.2335	0.1763	0.1529
Week 3	0.2873	0.2620	0.3010	0.2220	0.3042	0.3111
Week 4	0.2178	0.1545	0.1944	0.1268	0.1660	0.1496
Week 5	0.1914	0.1472	0.3022	NS	0.1277	0.1320
Week 6	0.0750	ND	0.0860	NS	0.0506	0.0315
Week 7	0.0583	0.1767	0.1292	NS	NS	0.0370
Week 8	0.0513	0.1324	0.0808	NS	NS	ND
Total Ca (mg)	1.4532	1.4190	1.5777	0.8308	1.0384	1.4599
Ca (µmol)	36.26	35.40	39.36	20.72	25.90	36.42

Table E-2 Total Ca in boitic elevated experiments

Not Sampled (NS) None Detected(ND) Harvested (H)

	Biotic Elevated												
	2	**3**	**5**	**6**	**8**	**9**	**11**	**12**	**14**	**15**	**16**	**17**	**18**
Week 1	0.6728	0.5925	0.7591	0.7313	0.6636	0.7045	0.4661	0.5761	1.2962	3.1143	1.5873	ND	0.2200
Week 2	0.3808	0.4405	0.2243	0.3807	0.2252	0.3516	0.4152	0.3785	0.1495	0.1621	0.1704	0.0952	0.2477
Week 3	0.7410	0.7472	0.4879	H	0.8127	1.1056	H	0.2793	H	0.1895	0.2096	0.3035	0.3441
Week 4	0.5340	0.3219	0.3331	H	0.3850	0.6338	H	0.2960	H	0.1244	0.1491	0.2060	0.1222
Week 5	0.4645	0.1665	H	H	0.9752	1.0901	H	H	H	H	0.1615	0.1584	H
Week 6	0.6521	0.0662	H	H	0.9825	1.4903	H	H	H	H	0.1708	0.1219	H
Week 7	1.4288	H	H	H	H	2.6427	H	H	H	H	0.2089	H	H
Week 8	1.5703	H	H	H	H	2.1601	H	H	H	H	0.3795	H	H
Plant content	0.6178	0.3133	0.4888	0.8097	0.0644	3.1397	0.3089	0.5368	0.2749	NS	1.0859	0.6462	0.4968
Total Ca (mg)	6.7709	2.3569	2.0020	1.6304	3.8173	13.0272	0.8989	1.7754	1.4294	3.2990	3.8318	1.2399	1.1396
Ca (µmol)	168.94	58.81	49.95	40.68	95.25	325.05	22.43	44.30	35.66	82.32	95.61	30.94	28.43

Table E-3 not E-1.. also no page number on this page.

Table E-1. Total Ca in biotic ambient conditions.

	Biotic Ambient									
	21	22	25	26	28	29	31	32	33	34
Week 1	1.6136	1.4467	0.5375	0.4728	0.3220	0.6619	0.5808	0.9617	0.4459	0.8107
Week 2	0.1212	0.1345	0.4435	0.3748	0.3043	0.3759	0.3934	0.2334	0.4057	0.3928
Week 3	H	0.3619	1.2917	0.7389	H	1.3272	0.6685	1.2286	H	0.1433
Week 4	H	0.1174	0.7388	0.9089	H	1.1810	0.3218	0.8124	H	0.4009
Week 5	H	H	0.9609	0.9856	H	H	0.5379	1.1357	H	H
Week 6	H	H	0.3470	0.2786	H	H	0.4617	0.5117	H	H
Week 7	H	H	H	ND	H	H	H	0.5309	H	H
Week 8	H	H	H	0.6398	H	H	H	0.3594	H	H
Plant content	0.1949	0.6867	0.6857	0.1632	0.3574	1.0119	0.0366	1.1634	0.5766	0.5911
Total Ca (mg)	1.6385	2.4559	4.7138	4.2714	0.6925	4.2666	2.7096	6.6461	1.1369	2.0476
Ca (µmol)	40.88	61.28	117.61	106.58	17.28	106.46	67.61	165.83	28.37	51.09

Appendix F Total P concentrations released with time.

Individual plant pots occupy separate columns.

Table F-1. Abiotic P concentrations

Not Sampled (NS) None Detected(ND) Harvested (H)

Mg P Leachate	*Abiotic Elevated*			*Abiotic Ambient*		
	1	*7*	*13*	*19*	*24*	*35*
Week 1	0.0005	0.0008	0.0036	ND	0.0033	0.0039
Week 2	0.0067	0.0013	0.0002	0.0217	0.0054	0.0008
Week 3	0.0389	0.0074	0.0077	0.0038	0.0075	0.0030
Week 4	0.0212	0.0037	0.0036	0.0034	0.0059	0.0034
Week 5	0.0021	0.0023	0.0030	H	0.0057	0.0016
Week 6	0.0025	0.0023	0.0011	H	0.0031	0.0019
Week 7	0.0178	0.0015	0.0015	H	0.0011	0.0007
Week 8	0.0013	0.0018	0.0228	H	0.0021	0.0014
Total P mg	0.0910	0.0210	0.0435	0.0289	0.0341	0.0167
P (µmol)	2.9367	0.6779	1.4032	0.9343	1.0999	0.5388

Table F-2. Total P released by biotic elevated experiments

Not Sampled (NS) None Detected(ND) Harvested (H)

	Biotic Elevated												
	2	**3**	**5**	**6**	**8**	**9**	**11**	**12**	**14**	**15**	**16**	**17**	**18**
Week 1	0.0668	0.0106	0.0734	0.0091	ND	0.0060	0.0018	0.0015	0.0242	0.0242	0.0242	0.0242	0.0242
Week 2	0.0684	0.0251	0.0114	0.0220	0.0031	0.0044	0.0242	0.0018	0.0097	0.0187	0.0123	0.0131	0.0154
Week 3	0.1782	0.0872	0.0385	H	0.0368	0.0112	H	0.0052	H	0.0219	0.0195	0.0518	0.0557
Week 4	0.0997	0.0342	0.0234	H	0.0082	0.0067	H	0.0028	H	0.0084	0.0064	0.0230	0.0189
Week 5	0.0444	0.0130	H	H	0.0149	0.0129	H	H	H	H	0.0033	0.0045	H
Week 6	0.0244	0.0578	H	H	0.0058	0.0079	H	H	H	H	0.0160	0.0093	H
Week 7	0.0304	H	H	H	0.0000	0.0062	H	H	H	H	0.0067	H	H
Week 8	0.0240	H	H	H	0.0000	0.0054	H	H	H	H	0.0186	H	H
Plant content	5.2888	1.7748	1.4596	3.0535	2.9448	10.4065	1.8811	2.6205	2.9180	NS	1.9600	1.4957	3.3949
Total P mg	5.8251	2.0029	1.6062	3.0846	3.0136	10.4672	1.9071	2.6317	2.9519	0.0732	2.0669	1.6216	3.5090
P (µmol)	188.06	64.66	51.86	99.59	97.29	337.94	61.57	84.96	95.30	2.36	66.73	52.35	113.29

Table F-3. Total P released from ambient experiments

Not Sampled (NS) None Detected(ND) Harvested (H)

Biotic Ambient

	21	22	25	26	28	29	*31*	*32*	*33*	*34*
Week 1	0.0131	0.0131	0.0314	0.0175	0.0002	0.0460	0.0044	0.0025	0.0016	0.0013
Week 2	0.0151	0.0064	0.0110	0.0288	0.0015	ND	0.0005	0.0008	0.0015	0.0007
Week 3	H	0.0161	0.1565	0.0822	H	0.0846	0.0230	0.0077	H	0.0020
Week 4	H	0.0083	0.0409	0.0550	H	0.1076	0.0010	ND	H	0.0079
Week 5	H	H	0.0431	0.0183	H	H	0.0206	0.0148	H	H
Week 6	H	H	0.0046	0.0032	H	H	0.0165	0.0027	H	H
Week 7	H	H	H	ND	H	H	H	0.0021	H	H
Week 8	H	H	H	0.0161	H	H	H	0.0064	H	H
Plant content	2.2805	1.9442	3.4175	3.5731	1.6577	2.7302	1.2867	5.1988	2.1561	3.1704
Total P (mg)	2.3087	1.9882	3.7049	3.7941	1.6594	2.9685	1.3527	5.2358	2.1591	3.1824
P (µmol)	74.5376	64.1883	119.6136	122.4936	53.5730	95.8372	43.6709	169.0380	69.7084	102.7436

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Table F-4. Ratio of Ca to P in shaker bath experiment

Ca (moles)	P (moles)	Ca:P	Time
1.17E-04	7.26E-06	16.04	10 days
1.06E-04	7.30E-06	14.53	10 days
1.17E-04	6.94E-06	16.78	20 days
1.17E-04	7.15E-06	16.30	20 days

endix G Log dissolution rates calculated based on Ca and P released.

Table G-1 Dissolution based on Ca released.

Biotic Elevated				Biotic Ambient				Abiotic Elevated				Abiotic Ambient			
Week 2	Ca (µ mol)	53.37	41.74	34.26	27.48	36.11	64.49	15.28	12.80	11.90		15.04	13.61	13.03	
Week 4	Ca (µ mol)	64.16	64.42	59.09	65.56	85.89	0.00	25.83	24.25	25.25		24.02	25.00	24.59	
Week 6	Ca (µ mol)	66.51	83.16	76.21	69.24	82.57	0.00	32.31	30.44	32.59		0.00	27.96	27.48	
Week 8	Ca (µ mol)	161.73	239.37	143.70	93.44	111.40	0.00	36.09	38.12	36.83		0.00	28.18	28.18	
Week 2	**Rate**	-11.63	-11.74	-11.83	-11.93	-11.81	-11.56	-12.20	-12.28	-12.31		-12.21	-12.25	-12.27	
Week 4	log(mol/cm2/sec)	-11.95	-11.94	-11.98	-12.01	-11.89		-12.33	-12.36	-12.34		-12.36	-12.35	-12.35	
Week 6		-12.11	-12.01	-12.05	-12.10	-12.03		-12.41	-12.43	-12.40			-12.47	-12.48	
Week 8		-11.91	-11.74	-11.96	-12.12	-12.05		-12.48	-12.46	-12.47			-12.59	-12.59	

Table G-2. Dissolution rates based on P released log(mols/cm^2/sec).

	Biotic Elevated			Biotic Ambient				Abiotic Elevated			Abiotic Ambient		
Week 2	P (µ mol)	99.97	62.20	95.20	74.54	53.99	70.08	0.27	0.09	0.06	0.82	0.29	0.14
Week 4	P (µ mol)	50.76	87.58	125.66	65.39	53.99	104.97	1.40	0.84	0.84	0.68	0.76	0.68
Week 6	P (µ mol)	63.30	99.40	53.08	45.39	113.80	0.00	1.19	1.18	1.14	0.00	0.92	0.89
Week 8	P (µ mol)	176.78	341.40	69.13	118.74	170.91	0.00	1.44	1.45	2.13	0.00	1.00	0.98
Week 2	**Rate**	-11.58	-11.79	-11.60	-11.72	-11.86	-11.74	-14.18	-14.64	-14.85	-13.70	-14.15	-14.45
Week 4	log(mol/cm^2/s	-12.27	-12.03	-11.88	-12.23	-12.31	-12.02	-13.82	-14.04	-14.04	-14.13	-14.08	-14.13
Week 6		-12.35	-12.16	-12.43	-12.51	-12.11		-14.07	-14.07	-14.08		-14.17	-14.19
Week 8		-12.09	-11.81	-12.50	-12.24	-12.08		-14.10	-14.10	-13.93		-14.26	-14.27

Appendix H . Stats

Table H-1. T-tests comparing average Ca released between biotic and abiotic experiments (α=0.05)

	Week 2		Week 4		Week 6		Week 8	
	Biotic	Abiotic	Biotic	Abiotic	Biotic	Abiotic	Biotic	Abiotic
Mean	2.07	0.55	2.73	0.99	3.03	1.26	6.07	1.48
Variance	0.51	0.02	0.14	0.02	0.09	0.03	6.86	0.01
Observations	6.00	6.00	6.00	6.00	5.00	5.00	4.00	3.00
df	5.00		7.00		0.06		3.00	
t Stat	5.12		10.48		8.00		3.50	
P(T<=t) one-tail	0.00		0.00		11.37		0.02	
t Critical one-tail	2.02		1.89		1.86		2.35	
P(T<=t) two-tail	0.00		0.00		0.00		0.04	
t Critical two-tail	2.57		2.36		2.31		3.18	

F-Test Two-Sample for Variances.

	Different		Different		Same		Different	
	Biotic	Abiotic	Biotic	Abiotic	Biotic	Abiotic	Biotic	Abiotic
Mean	2.07	0.55	2.73	0.99	3.03	1.26	6.07	1.48
Variance	0.51	0.02	0.14	0.02	0.09	0.03	6.86	0.01
Observations	6.00	6.00	6.00	6.00	5.00	5.00	4.00	3.00
df	5.00	5.00	5.00	5.00	4.00	4.00	3.00	2.00
F	26.31		5.71		3.15		983.39	
P(F<=f) one-tail	0.00		0.04		0.15		0.00	
F Critical one-tail	5.05		5.05		6.39		19.16	

Table H-2. T-test comparing total P released between biotic and abiotic experiments (α=0.05)

	Week 2		*Week 4*		*Week 6*		*Week 8*	
	Biotic	Abiotic	Biotic	Abiotic	Biotic	Abiotic	Biotic	Abiotic
Mean	2.35	0.01	2.66	0.03	2.30	0.03	6.70	0.04
Variance	0.32	0.00	0.54	0.00	0.90	0.00	6.66	0.00
Observations	6.00	6.00	6.00	6.00	5.00	5.00	4.00	5.00
df	5.00		5.00		4.00		3.00	
t Stat	10.19		8.77		5.34		5.16	
P(T<=t) one-tail	0.00		0.00		0.00		0.01	
t Critical one-tail	2.02		2.02		2.13		2.35	
P(T<=t) two-tail	0.00		0.00		0.01		0.01	
t Critical two-tail	2.57		2.57		2.78		3.18	

F-Test Two-Sample for Variances.

			Different		Different			Different
	Biotic	Abiotic	Biotic	Abiotic	Biotic	Abiotic	Biotic	Abiotic
Mean	2.35	0.01	2.66	0.03	2.30	0.03	6.70	0.04
Variance	0.32	0.00	0.54	0.00	0.90	0.00	6.66	0.00
Observations	6.00	6.00	6.00	6.00	5.00	5.00	4.00	5.00
df	5.00	5.00	5.00	5.00	4.00	4.00	3.00	4.00
F	4567.37		6916.95		33674.0		29600.0	
P(F<=f) one-tail	0.00		0.00		0.00		0.00	
F Critical one-tail	5.05		5.05		6.39		6.59	

Table H-3. T-test comparing average plant weight (g) between elevated and ambient conditions (α=0.05).

	Week 2		*Week 8*		*Week 6*		*Week 8*	
	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient
Mean	0.38	0.32	0.32	0.48	0.61	0.78	2.24	1.45
Variance	0.01	0.00	0.01	0.06	0.40	0.25	2.27	0.03
Observations	3.00	3.00	3.00	3.00	3.00	2.00	2.00	2.00
df	3.00		0.03		3.00		1.15	
t Stat	1.14		4.00		-0.33		2.00	
P(T<=t) one-tail	0.17		-1.08		0.38		0.73	
t Critical one-tail	2.35		2.13		2.35		2.92	
P(T<=t) two-tail	0.34		0.34		0.76		0.54	
t Critical two-tail	3.18		2.78		3.18		4.30	

F-Test Two-Sample for Variances

Different

	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient
Mean	0.38	0.32	0.48	0.32	0.78	0.61	1.59	1.45
Variance	0.01	0.00	0.06	0.01	0.25	0.40	2.37	0.03
Observations	3.00	3.00	3.00	3.00	2.00	3.00	3.00	2.00
df	2.00	2.00	2.00	2.00	1.00	2.00	2.00	1.00
F	7.28		10.83		0.62		79.30	
P(F<=f) one-tail	0.12		0.08		0.49		0.08	
F Critical one-tail	19.00		19.00		0.01		199.50	

Table H-4. T-test comparing average Ca content (mg) of leachate in ambient and elevated biotic experiments (α=0.1).

	Week 1		*Week 2*		*Week 3*		*Week 4*	
	Elevated	*Ambient*	*Elevated*	*Ambient*	*Elevated*	*Ambient*	*Elevated*	*Ambient*
Mean	0.95	0.79	1.23	1.10	1.75	1.82	2.06	2.29
Variance	0.60	0.19	0.01	0.01	0.10	0.28	0.03	0.13
Observations	12.00	10.00	13.00	10.00	10.00	8.00	10.00	7.00
Pooled Variance	0.41		0.01		0.18		0.07	
df	20.00		21.00		16.00		15.00	
t Stat	0.59		2.53		-0.37		-1.83	
P(T<=t) one-tail	0.28		0.01		0.36		0.04	
t Critical one-tail	1.33		1.32		1.34		1.34	
P(T<=t) two-tail	0.56		0.02		0.71		0.09	
t Critical two-tail	1.72		1.72		1.75		1.75	

	Week 5		*Week 6*		*Week 7*		*Week 8*	
	Elevated	*Ambient*	*Elevated*	*Ambient*	*Elevated*	*Ambient*	*Elevated*	*Ambient*
Mean	2.29	2.73	2.87	2.68	4.29	2.94	5.66	3.44
Variance	0.19	0.21	0.33	0.01	1.48	0.14	0.82	0.04
Observations	7.00	5.00	6.00	4.00	3.00	2.00	3.00	2.00
Pooled Variance	0.20		0.21		1.03		0.56	
df	10.00		8.00		3.00		3.00	
t Stat	-1.71		0.65		1.46		3.25	
P(T<=t) one-tail	0.06		0.27		0.12		0.02	
t Critical one-tail	1.37		1.40		1.64		1.64	
P(T<=t) two-tail	0.12		0.54		0.24		0.05	
t Critical two-tail	1.81		1.86		2.35		2.35	

Table H-5. T-test comparing average weekly P content of leachate in elevated and ambient biotic experiments (α=0.1).

	Week 1		Week		Week 3		Week 4	
	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient
Mean	0.02	0.01	0.04	0.02	0.09	0.07	0.11	0.10
Variance	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Observations	13.00	10.00	13.00	10.00	10.00	7.00	10.00	7.00
Pooled Variance	0.00		0.00		0.00		0.00	
df	21.00		21.00		15.00		15.00	
t Stat	1.34		3.27		0.65		0.53	
P(T<=t) one-tail	0.10		0.00		0.26		0.30	
t Critical one-tail	1.32		1.32		1.34		1.34	
P(T<=t) two-tail	0.19		0.00		0.53		0.60	
t Critical two-tail	1.72		1.72		1.75		1.75	

	Week 5		Week 6		Week 7		Week 8	
	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient
Mean	0.13	0.13	0.15	0.11	0.11	0.09	0.18	0.10
Variance	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Observations	6.00	4.00	6.00	4.00	5.00	2.00	3.00	2.00
Pooled Variance	0.00		0.00		0.01		0.00	
df	8.00		8.00		5.00		3.00	
t Stat	0.01		3.79		0.28		9.94	
P(T<=t) one-tail	0.50		0.00		0.40		0.00	
t Critical one-tail	1.40		1.40		1.48		1.64	
P(T<=t) two-tail	0.99		0.01		0.79		0.00	
t Critical two-tail	1.86		1.86		2.02		2.35	

Table H-6. T- test comparing average pH between abiotic and biotic experiments (α=0.05).

	Week 1		*Week 2*		*Week 3*		*Week 4*	
	Abiotic	*Biotic*	*Abiotic*	*Biotic*	*Abiotic*	*Biotic*	*Abiotic*	*Biotic*
Mean	4.87	4.78	5.14	4.87	5.05	4.89	4.92	4.85
Variance	0.56	0.75	0.37	0.15	0.14	0.22	0.19	0.34
Observations	6.00	28.00	6.00	28.00	6.00	19.00	6.00	17.00
Pooled Variance	0.72		0.18		0.20		0.30	
df	32.00		32.00		23.00		21.00	
t Stat	0.22		1.37		0.76		0.26	
P(T<=t) one-tail	0.41		0.09		0.23		0.40	
t Critical one-tail	1.69		1.69		1.71		1.72	
P(T<=t) two-tail	0.83		0.18		0.45		0.80	
t Critical two-tail	2.04		2.04		2.07		2.08	

	Week 5		*Week 6*		*Week 7*		*Week 8*	
	Abiotic	*Biotic*	*Abiotic*	*Biotic*	*Abiotic*	*Biotic*	*Abiotic*	*Biotic*
Mean	5.18	4.76	5.33	4.89	5.17	4.98	5.33	4.38
Variance	0.14	0.19	0.13	0.54	0.19	1.04	0.34	0.31
Observations	5.00	10.00	5.00	10.00	5.00	5.00	5.00	5.00
Pooled Variance	0.17		0.41		0.62		0.32	
df	13.00		13.00		8.00		8.00	
t Stat	1.84		1.25		0.38		2.64	
P(T<=t) one-tail	0.04		0.12		0.36		0.01	
t Critical one-tail	1.77		1.77		1.86		1.86	
P(T<=t) two-tail	0.09		0.23		0.71		0.03	
t Critical two-tail	2.16		2.16		2.31		2.31	

Table H-7. T-test comparing average pH between elevated and ambient experiments (α=0.05).

	Week 1		*Week 2*		*Week 3*		*Week 4*	
	Elevated	*Ambient*	*Elevated*	*Ambient*	*Elevated*	*Ambient*	*Elevated*	*Ambient*
Mean	4.57	4.99	4.88	4.91	4.96	4.80	4.94	4.76
Variance	0.65	0.81	0.13	0.18	0.28	0.15	0.53	0.14
Observations	14.00	14.00	12.00	14.00	11.00	8.00	9.00	8.00
Pooled Variance	0.73		0.16		0.22		0.35	
df	26.00		24.00		17.00		15.00	
t Stat	-1.31		-0.21		0.77		0.65	
P(T<=t) one-tail	0.10		0.42		0.23		0.26	
t Critical one-tail	1.06		1.06		1.07		1.34	
P(T<=t) two-tail	0.20		0.84		0.45		0.53	
t Critical two-tail	1.48		1.49		1.51		1.75	

	Week 5		*Week 6*		*Week 7*		*Week 8*	
	Elevated	*Ambient*	*Elevated*	*Ambient*	*Elevated*	*Ambient*	*Elevated*	*Ambient*
Mean	4.57	5.03	4.79	5.05	4.27	6.03	4.04	4.89
Variance	0.17	0.12	0.91	0.04	0.16	0.15	0.12	0.13
Observations	6.00	4.00	6.00	4.00	3.00	2.00	3.00	2.00
Pooled Variance	0.15		0.58		0.16		0.12	
df	8.00		8.00		3.00		3.00	
t Stat	-1.84		-0.54		-4.84		-2.63	
P(T<=t) one-tail	0.05		0.30		0.01		0.04	
t Critical one-tail	1.40		1.40		1.64		1.64	
P(T<=t) two-tail	0.10		0.60		0.02		0.08	
t Critical two-tail	1.86		1.86		2.35		2.35	

Table H-8. T-tests comparing total Ca released (α=0.1).

	Week 2		*Week 4*		*Week 6*		*Week 8*	
	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient
Mean	1.73	2.41	2.51	2.96	3.02	3.04	8.04	4.10
Variance	0.15	0.78	0.01	0.18	0.11	0.14	4.84	0.26
Observations	3.00	3.00	3.00	3.00	3.00	2.00	2.00	2.00
Pooled Variance	0.46		0.10		0.12		2.55	
df	4.00		4.00		3.00		2.00	
t Stat	-1.22		-1.78		-0.08		2.46	
P(T<=t) one-tail	0.15		0.08		0.47		0.07	
t Critical one-tail	1.53		1.53		1.64		1.89	
P(T<=t) two-tail	0.29		0.15		0.94		0.13	
t Critical two-tail	2.13		2.13		2.35		2.92	

Table H-9. T-test comparing total P released from biotic elevated and biotic ambient experiments (α=0.1).

	Week 2		*Week 4*		*Week 6*		*Week 8*	
	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient
Mean	2.66	2.05	2.60	2.73	2.19	2.46	8.03	5.38
Variance	0.41	0.11	0.94	0.40	0.64	2.24	13.00	0.02
Observations	3.00	3.00	3.00	3.00	3.00	2.00	2.00	2.00
Pooled Variance	0.26		0.67		1.17		6.51	
df	4.00		4.00		3.00		2.00	
t Stat	1.46		-0.20		-0.28		1.04	
P(T<=t) one-tail	0.11		0.43		0.40		0.20	
t Critical one-tail	1.53		1.53		1.64		1.89	
P(T<=t) two-tail	0.22		0.85		0.80		0.41	
t Critical two-tail	2.13		2.13		2.35		2.92	

Table H-10. T-test Comparing rates based on total Ca released. (α=0.1).

	Week 2		*Week 4*		*Week 6*		*Week 8*	
	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient
Mean	1.49213E-12	1.55042E-12	7.46907E-13	1.11683E-12	7.37043E-13	1.07203E-12	1.89539E-12	1.10895E-12
Variance	1.68264E-25	2.57126E-25	3.88611E-26	1.96123E-25	1.42184E-25	1.62681E-25	7.09571E-25	1.14052E-25
Observations	3	3	3	3	3	2	2	2
Pooled Variance	2.12695E-25		1.17492E-25		1.49017E-25		4.11811E-25	
df	4		4		3		2	
t Stat	-0.15		-1.32		-0.95		1.23	
P(T<=t) one-tail	0.44		0.13		0.21		0.17	
t Critical one-tail	1.53		1.53		1.64		1.89	
P(T<=t) two-tail	0.88		0.26		0.41		0.35	
t Critical two-tail	2.13		2.13		2.35		2.92	

Table H-11. T-test comparing rates calculated based on total P released (α=0.1).

	Week 2		**Week 4**		**Week 6**		**Week 8**	
	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient
Mean	2.22454E-12	2.06876E-12	8.81141E-13	7.8788E-13	4.99319E-13	5.58745E-13	1.20422E-12	7.04974E-13
Variance	2.90269E-25	7.76145E-26	1.03661E-25	3.55722E-26	2.72841E-26	1.35064E-25	2.35458E-25	2.53361E-26
Observations	3	3	3	3	3	2	2	2
Pooled Variance	1.83942E-25		6.96166E-26		6.32107E-26		1.30397E-25	
df	4		4		3		2	
t Stat	0.44		0.43		-0.26		1.38	
P(T<=t) one-tail	0.34		0.34		0.41		0.15	
t Critical one-tail	1.53		1.53		1.64		1.89	
P(T<=t) two-tail	0.68		0.69		0.81		0.30	
t Critical two-tail	2.13		2.13		2.35		2.92	

Table H-12. T-test comparing rate of dissolution based on Ca and P released at week 8 (α=0.05).

	Rate[Ca]	*Rate [P]*
Mean	1.20422E-12	1.89539E-12
Variance	2.35458E-25	7.09571E-25
Observations	2	2
Pooled Variance	4.72514E-25	
Hypothesized Mean Difference	0	
df	2	
t Stat	-1.005487008	
P(T<=t) one-tail	0.210271784	
t Critical one-tail	1.885618083	
P(T<=t) two-tail	0.420543567	
t Critical two-tail	2.91998558	

Appendix I Images from experiments

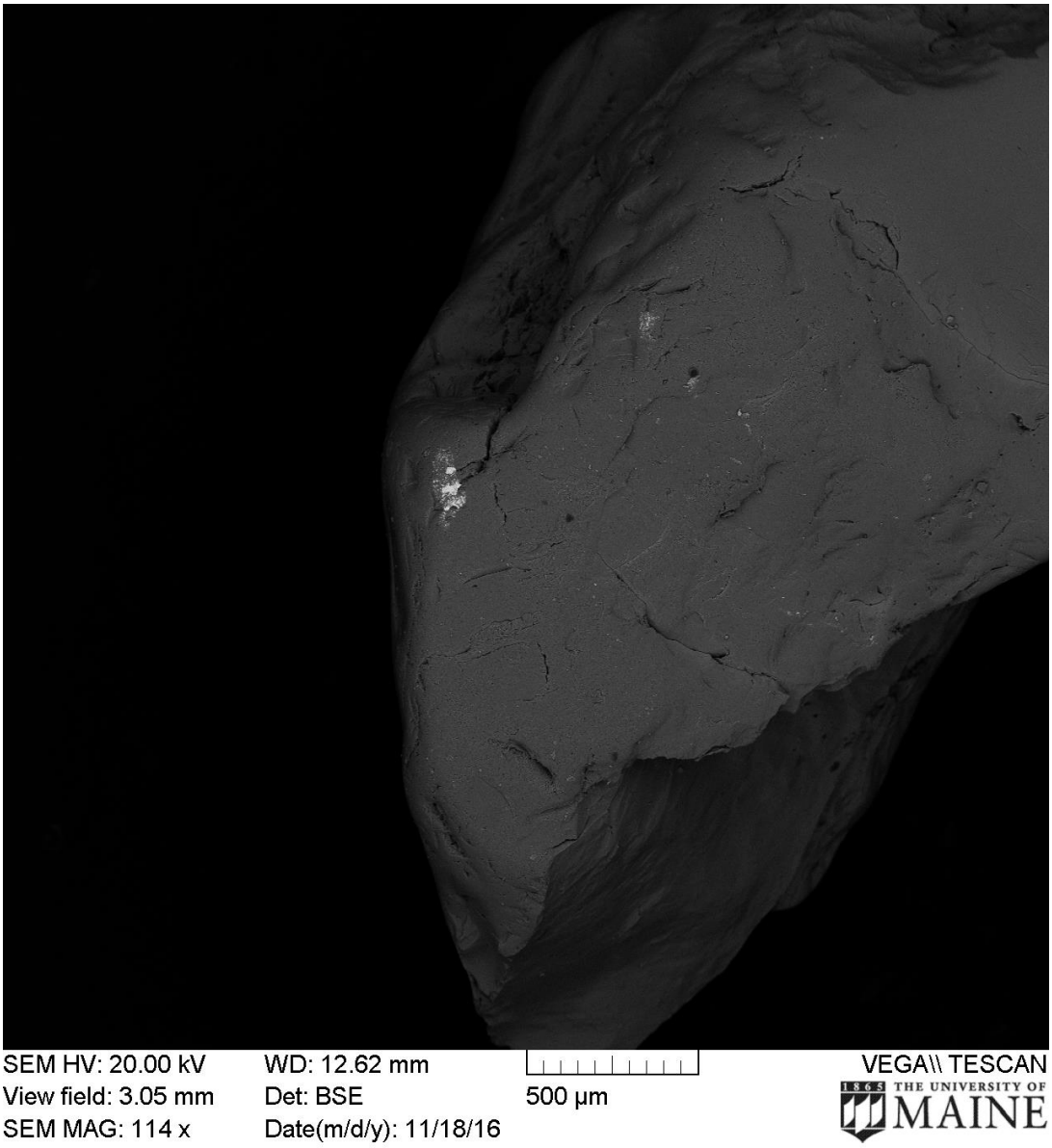

Figure I-1 Fe precipitates on the surface of a quartz grain

Figure I-2 characteristic stunted leaf growth and hooked leaf tips of plants deficient in Zn.

Figure I-3. Plant pot used in experiment

Figure I-4 Image showing experimental growth chamber

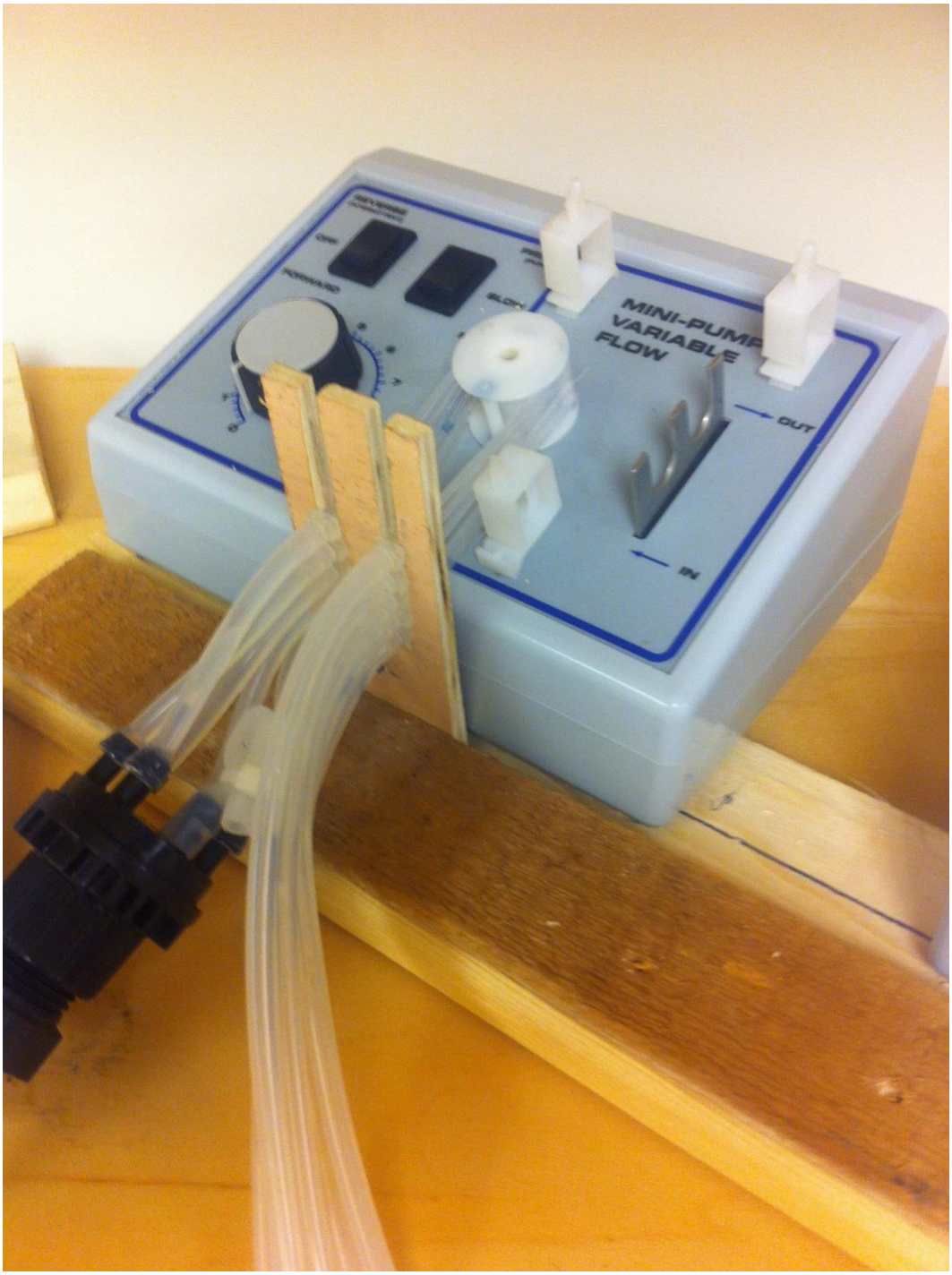

Figure I-5 Single channel peristaltic pump set up to run 5 tubes

BIOGRAPHY OF THE AUTHOR

Brian Morra was born one cold morning in March. The year was 1990 and a light rain wetted the farmhouse where he was delivered by young midwife. Forty-eight hours later he had bedded her and started working the fields. Helping his father harvest grains he quickly realized he was destined to become something great. It was this rugged upbringing that sculpted his voracious curiosity of the natural world and his bodacious 8-pack.

At age 12 he took himself on a walkabout in the Australian Bush. During this time, he lived off the land and consumed mostly insects and rodent meat he hunted for. These were times for reflection on man's impact on the environment.

Following these colorful experiences Brian studied geology at the University of Idaho and completed his B.S. in the spring of 2012. After 2 years working for the Peace Corps in Peru he moved to Maine to begin his graduate studies. Brian is a candidate for the Master of Science degree in Earth and Climate Sciences from the University of Maine in May 2017.