Wilfrid Laurier University

Scholars Commons @ Laurier

Theses and Dissertations (Comprehensive)

2019

Context is everything: an investigation of Spanish River Carbonatite and its effects on soil-plant-microorganism systems

James MC Jones
Wilfrid Laurier University, jone3630@mylaurier.ca

Follow this and additional works at: https://scholars.wlu.ca/etd

Part of the Agricultural Science Commons, Environmental Microbiology and Microbial Ecology Commons, Integrative Biology Commons, Plant Biology Commons, and the Systems Biology Commons

Recommended Citation

Jones, James MC, "Context is everything: an investigation of Spanish River Carbonatite and its effects on soil-plant-microorganism systems" (2019). *Theses and Dissertations (Comprehensive)*. 2212.
https://scholars.wlu.ca/etd/2212

This Dissertation is brought to you for free and open access by Scholars Commons @ Laurier. It has been accepted for inclusion in Theses and Dissertations (Comprehensive) by an authorized administrator of Scholars Commons @ Laurier. For more information, please contact scholarscommons@wlu.ca.

Context is everything: an investigation of Spanish River Carbonatite and its effects on soil-plant-microorganism systems

by

James M.C. Jones

BSc - Biochemistry and Molecular Biology, UNBC, 2013

MSc – Integrative Biology, WLU, 2015

DISSERTATION

Submitted to the Biological and Chemical Sciences Program

Faculty of Science

in partial fulfillment of the requirements for the

Doctor of Philosophy in the Biological and Chemical Sciences

Wilfrid Laurier University

2019

James M.C. Jones 2019©

Abstract:

With growing concerns about agricultural sustainability and food security, the use of rock fertilizers and agrominerals is receiving renewed interest. A wide variety of geological resources have been proposed as crop nutrient sources, with silicate rocks the predominant focus. Carbonatite rocks are known to weather more readily than silicate rocks; yet, they have received relatively little attention as it is thought their high Ca and Mg contents hinder effective nutrient release. However, there is strong evidence that the nutrients within carbonatite rocks are easily accessible to plants, and that these rocks have noticable effects on crop plant growth. Here I propose a framework to understand the mode of action of carbonatites on soil fertility and plant nutrition by integrating research at multiple scales, i.e., from individual plants to the ecosystem, including soil microorganisms. The model stems from greenhouse experiments on two crops, pea and wheat, and an extensive survey of the carbonatite deposit. It is emphasized that a systems-approach must be taken when examining carbonatites as their effects are strongly context-dependent, and there is evidence that a three-way interaction between plant-carbonatite-microorganisms is responsible for some of the observed effects on plants. The framework presented is intended not only to synthesize the current knowledge on carbonatites as rock fertilizers but also to guide future research on this and other similar geological resources.

Acknowledgements:

It is no small feat to complete a Ph.D. dissertation, and there are many people who helped me along the way whom I wish to thank.

Foremost among these are my supervisors, Drs. Frédérique Guinel and Pedro M. Antunes. Throughout the Ph.D. process, I have been able to rely upon them for help with experimental design, with interpreting the (sometimes confusing!) results, and especially with editing my writing! There is no doubt that without their support, this work would not have been possible. Thank you both for your determination and confidence in my ability to succeed!

Although he was not able to see the end of my journey, Dr. Alizera Navabi was instrumental in helping to shape the experiments I did with wheat. Indeed, it was largely because of the differences between wheat and pea in responding to SRC that I was able to develop the model for carbonatite mechanism of actions. He was always willing to meet with me and share his thoughts through his encouraging and inspiring attitude towards academic research. He will be sorely missed.

No human is an island, and it is through our interactions with friends and family that we are made stronger. Through thick and thin, my friends in Ontario and in British Columbia were there for me. My family was also very supportive, and I am grateful for the care packages and visits back to BC that they provided. I would also like to show my appreciation for Rey Cogswell, who has had my back throughout the Ph.D. these past four years.

The work here was a continuation of a project started during my Masters, also on Spanish River Carbonatite (SRC). I originally expected to work on plant hormones and biochemistry when I first made the journey to Ontario, but fate (and Oleg Stukalov!) had other plans. After meeting with a local geologist and farmer, John Slack, we began to look more closely at this curious SRC rock, and so began six years of research. I am grateful to both Oleg and John for their inspiration to start this research, and for motivating me to look more at sustainability in agriculture.

Finally, I acknowledge that Boreal Agrominerals provided funding and materials for the project. Additional funding was provided by NSERC, the Ontario Graduate Scholarship (OGS), and the Ontario Centres of Excellence (OCE).

Table of Contents

Chapter 1 - General introduction 1

Chapter 2 - Carbonatites as rock fertilizers 6

Chapter 3 - Does a carbonatite deposit influence its surrounding ecosystem? 37

Chapter 3 - Supplemental data 63

Chapter 4 - Carbonatite enhances growth of plants through their nutrient-foraging strategy ... 67

Chapter 4 - Supplemental data 111

Chapter 5 - Carbonatite mechanism of action and context-dependence 117

List of Tables:

Table 2.1: Non-exhaustive list of carbonattite-associated minerals following Heinrich (1980)..... 17
Table 3.1: Mean soil pH for the major soil horizons (A, B, C) separated by SRC-influence 49
Table 3.2: Bacterial OTUs of differential abundance between high and low/negligible SRC sites..... 53
Table 4.1: Plant parameters from 28 day-old nodulated pea (nodulation experiment) 83
Table 4.2: Plant parameters from 14 day-old pea (early growth nutrient experiment) 85
Table 4.3: Plant parameters from 21 day-old pea (microbial addition experiment)..... 89
Table 4.4: Plant parameters from 56 day-old wheat (SRC response experiment). 96
Table 4.5: Plant paremeters from 14 day-old wheat (early growth nutrient experiment). 100
Table 4.6: Brief summary of key experimental findings..... 101

List of Figures and Illustrations:

Figure 2.1: Components of a simple agroecosystem. 10
Figure 2.2: A few of the key interactions in a simple three-component agroecosystem 14
Figure 2.3: Key direct and indirect factors influencing mineral weathering..... 27
Figure 3.1: Overview of the Spanish River Carbonatite (SRC) deposit 42
Figure 3.2: Principal component analysis of soil chemistry factors coloured by zone 47
Figure 3.3: Mean soil chemistry values across zones and SRC-influence categories..... 48
Figure 3.4: Plant community responses across SRC-influence categories 50
Figure 3.5: Bacterial and fungal community responses across SRC-influence categories. 52
Figure 4.1: Shoot and root biomass of 28 day-old inoculated peas (nodulation experiment)..... 82
Figure 4.2: Mean nutrient content of 28 day-old pea (nodulation experiment) 84
Figure 4.3: Shoot and root biomass of 14 day-old pea (early growth nutrient experiment). 86
Figure 4.4: Shoot and root biomass of 21 day-old pea (microbial addition experiment)..... 88
Figure 4.5: Shoot nutrient content 21 day-old pooled pea shoots (microbial addition experiment).... 90
Figure 4.6: Specific root length and heterotrophic CFU from 21 day-old pea 91
Figure 4.7: Weekly shoot height of wheat over the eight-week growing period for trial two. 94
Figure 4.8: Shoot and root biomass and representative root system scans of 56 day-old wheat. 95
Figure 4.9: Average shoot nutrient contents of 56 day-old wheat 97
Figure 4.10: Linear regression analysis of SRL and shoot nutrient content in 56 day-old wheat..... 99
Figure 5.1: Working model of how SRC is expected to alter agroecosystems 124
Figure 5.2: Examples of the disciplines involved in researching the effects of carbonatite rock 130

Chapter 1 - General introduction

The sustainability of agriculture is something often taken for granted by those not directly involved with it. One generally expects that food will be available for purchase tomorrow, much as it was in the days before. However, modern agriculture faces a number of issues that threaten the sustainability of crop production in the years to come. A few of these issues, in no particular order, include the current and future impacts of climate change on cropping systems (Campbell et al. 2016; Altieri and Nicholls 2017), the increasing global human population (Alexandratos 2005), the dwindling reserves of resources for production of fertilizers (e.g., in phosphate resources; Cordell et al. (2009), and the stability of the soils in which crop production takes place (e.g., soil degradation and nutrient depletion; van Straaten (2007). There is no single solution to address the sustainability of agriculture because of the numerous components that make up even a "simple" agroecosystem. Using the definition of Conway (1987) and Gliessman (2004), an agroecosystem is an ecosystem which has become managed by humans for the production of agricultural goods. The components of an agroecosystem are often simplified to three components: the growing medium (usually soil and the nutrients it contains), the climate (photoperiod, temperatures, precipitation, etc.), and the plants or crops to be grown. This simplification ignores a number of important factors, such as the other inhabitants of the soils (e.g., microorganisms) and the social factors related to farming practices (e.g., the cost of a given crop or piece of farming equipment). In order to overcome the issues hindering sustainable agricultural practices, a systems approach is needed with the employment of both new tools and new understanding.

One tool that may prove useful in achieving sustainable agriculture is the use of geological resources ("agrogeology"; van Straaten 2007). Although their use in agriculture is not new, geological resources as nutrient sources have largely been displaced by highly-soluble alternatives. Thus, a full comprehension of their effects on agroecosystems is lacking. This is especially obvious in the case of carbonatites, a type of carbonate-rich igneous rock with high relative solubility compared to other rock types but with minimal research on their agricultural applications. The research that has been

conducted with carbonatites demonstrates that they can be highly effective as nutrient sources for plants (e.g., Bakken et al. 1997a, 1997b), but that this effectiveness seems to be context-dependent. Furthermore, it is unclear to what extent carbonatites interact with other agroecosystem components beyond plants, e.g., microorganisms. In agrogeology, an emphasis is placed on the use of local resources to minimize environmental impact, so here I have focused on one specific carbonatite from a deposit located near Sudbury, ON, Canada. It is known as Spanish River Carbonatite (SRC), and although it has been sold as an agricultural amendment for many years, a description of its effects on agroecosystems is lacking.

In this dissertation, a systems-approach was taken to study SRC in order to characterize its effects on plants, soils and soil microorganisms. The overall research aims were to clarify whether SRC would provide benefits to agroecosystems, to identify how these benefits would be imparted (i.e., a mechanism of action), and to understand under which contexts these benefits could be realized. These objectives were completed in three parts with: I) a review of carbonatites as rock fertilizers (Chapter 2), II) a survey of the SRC deposit to assess whether the overlying ecosystem was affected by the presence of the carbonatite (Chapter 3), and III) a determination of the effects and mechanism of action of SRC on pea and wheat (Chapter 4). The results from these sections have been integrated into a descriptive model of carbonatite rock fertilizers placed into the context of the agroecosystem (Chapter 5).

Chapter 2 - Carbonatites as rock fertilizers (literature review)

A challenge for all forms of agriculture is the consistent and efficient delivery of nutrients to sustain plant growth. Rock fertilizers and agrominerals have been proposed as an alternative to water-soluble fertilizers because they are thought to be less prone to nutrient leaching and runoff (Bakken et al. 1997a; van Straaten 2007). Geological resources chosen for their nutrient content, rock fertilizers or agrominerals are thought to provide nutrients on-demand to plants and thereby mitigate the environmental issues associated with high-mobility nutrient sources (van Straaten 2006). However,

because of the societal focus on yields obtained with highly-efficient soluble fertilizers, there are a number of unanswered questions regarding the type, effectiveness, and ultimate usefulness of rock fertilizers and agrominerals. Furthermore, the inherently low solubility of most geological resources means that they may not provide sufficient nutrients to justify their use. Carbonatites are predominantly comprised of carbonate minerals which are known to weather relatively quickly compared to other mineral types; this gives them obvious appeal as rock fertilizers. Despite this appeal, research is sparse and many questions on their efficacy and on the optimal conditions for their use remain open. In this chapter, I address these knowledge gaps starting with an introduction to the topic of rock fertilizers and agrominerals, followed by reviews of the geology and mineralogy of carbonatites, and the agricultural use of carbonatites. I integrate factors known to affect mineral weathering into an agricultural context to inform the effective use of carbonatites or similar rock fertilizers.

Chapter 3 - Does a carbonatite deposit influence its overlying ecosystem?

Although there are indications that carbonatites have effects on plants *in situ*, it is unclear to what extent their long-term presence in deposits can influence the overlying ecosystems. Here, an ecological survey was conducted to determine whether the soil chemistry, the plant communities, and the soil microbial communities were being impacted by the Spanish River Carbonatite deposit. Because the deposit consists of several zones of variable mineralogy, it was expected that a zone-specific signature would be identifiable in the measured ecosystem parameters.

Chapter 4 - Characterizing the effects of a carbonatite rock fertilizer on two crop plant species, pea and wheat.

In previous work aimed at assessing the effects of SRC on the growth and development of pea (Jones 2016), several distinct effects attributed to the carbonatite were found. However, it was unclear

what was causing these effects, and especially whether they were simply a function of increased pH. In this chapter, I used a simplistic three-component system (substrates, plants, microorganisms) to expand upon and clarify these findings. Two crop plants were tested, pea (*Pisum sativum* L.) and wheat (*Triticum aestivum* L.). In particular, SRC was compared against calcitic lime to identify whether the effects seen with SRC depended on increases in substrate pH. While lime and SRC share broad mineralogical similarity (i.e., having high percentages of calcite), it was expected that the diverse mineralogical composition of SRC would impart effects on plants and microorganisms that were distinct from those of lime.

Chapter 5 - Integration of results into a descriptive model of carbonatite rock fertilizers

With the data gained during the past four years, I created a working model illustrating the influence of carbonatites on plants and other agroecosystem components, and this model is presented in this chapter. Reflecting the work done in the dissertation, the model covers the agroecosystem level (extrapolating from the SRC deposit survey), the individual plant level (incorporating the work done with pea and wheat), and finally the microbial level (based on both field and laboratory findings). It is intended that this model will serve to inform current use of carbonatites as rock fertilizers and also guide future research. Furthermore, because there is strong interest among farmers on how to use SRC and other rock fertilizers, it is my suggestion that this future research takes place in partnership with those who would benefit directly from the findings.

Literature cited:

Alexandratos, N. 2005. Countries with rapid population growth and resource constraints: Issues of food, agriculture, and development. Popul. Dev. Rev. **31**(2): 237–258. doi:10.1111/j.1728-4457.2005.00064.x.

Altieri, M.A., and Nicholls, C.I. 2017. The adaptation and mitigation potential of traditional agriculture in a changing climate. Clim. Change **140**: 33–45. doi:10.1007/s10584-013-0909-y.

Bakken, A.K., Gautneb, H., and Myhr, K. 1997a. Plant available potassium in rocks and mine tailings with biotite, nepheline and K-feldspar as K-bearing minerals. Acta Agric. Scand. Sect. B - Soil Plant Sci. **47**(3): 129–134. doi:10.1080/09064719709362452.

Bakken, A.K., Gautneb, H., and Myhr, K. 1997b. The potential of crushed rocks and mine tailings as slow-releasing K fertilizers assessed by intensive cropping with Italian ryegrass in different soil types. Nutr. Cycl. Agroecosystems **47**: 41–48. doi:10.1007/BF01985717.

Campbell, B.M., Vermeulen, S.J., Aggarwal, P.K., Corner-Dolloff, C., Girvetz, E., Loboguerrero, A.M., Ramirez-Villegas, J., Rosenstock, T., Sebastian, L., Thornton, P.K., and Wollenberg, E. 2016. Reducing risks to food security from climate change. Glob. Food Sec. **11**: 34–43. doi:10.1016/j.gfs.2016.06.002.

Conway, G.R. 1987. The properties of agroecosystems. Agric. Syst. **24**(2): 95–117. doi:10.1016/0308-521X(87)90056-4.

Cordell, D., Drangert, J.-O., and White, S. 2009. The story of phosphorus: Global food security and food for thought. Glob. Environ. Chang. **19**(2): 292–305. doi:10.1016/j.gloenvcha.2008.10.009.

Gliessman, S.R. 2004. Agroecology and Agroecosystems. *In* Agroecosystems Analysis. *Edited by* R. Diane and C. Francis. Agron. Monogr. 43. ASA, CSSA, SSSA, Madison, WI. p. 11. doi:10.2134/agronmonogr43.frontmatter.

Jones, J.M. 2016. Spanish River Carbonatite: Its benefits and potential use as a soil supplement in agriculture. Wilfrid Laurier University. Available from https://scholars.wlu.ca/etd/1795.

van Straaten, P. 2006. Farming with rocks and minerals: challenges and opportunities. An. Acad. Bras. Cienc. **78**(4): 731–747. doi:10.1590/S0001-37652006000400009.

van Straaten, P. 2007. Agrogeology: The use of rocks for crops. Enviroquest Ltd., Toronto, CA.

Chapter 2 - Carbonatites as rock fertilizers

This chapter is intended to be published as a literature review which covers the role of carbonatites as rock fertilizers. It also details several key factors which influence mineral weathering, and how these relate to the predicted effectiveness of carbonatites.

Carbonatites as rock fertilizers: a review

Jones JMC[1], Antunes PM[2], Guinel FC[1].

1 – Department of Biology, Wilfrid Laurier University, Waterloo, Ontario, Canada

2 – Department of Biology, Algoma University, Sault Ste. Marie, Ontario, Canada

Abstract:

Rock fertilizers are geological resources used in agriculture for their nutrient content, but their slow weathering rates hinder their effectiveness. Carbonatites, igneous rocks with high carbonate and low silicate mineral composition, have a high relative weathering rate and often contain nutrient-bearing accessory minerals (e.g., apatite and biotite). However, few studies have explored their application in agroecosystems and often with limited scope. Here I evaluate plant responses to carbonatite additions to soil from a systems-level perspective and take into consideration factors that affect mineral weathering. The effectiveness of carbonatites depends on the interactions of many components (e.g., soils, plants, microorganisms), and these interactions need to be accounted for when carbonatites are used in agricultural or research settings.

Key words: sustainable agriculture, agrogeology, plant-mineral interactions, microbe-mineral interactions

Introduction

The use of rocks and minerals in agriculture is not a new concept, and it has been the focus of increased investigation to address agricultural challenges in sustainability. Geological resources which contain nutrients that plants require and can access are considered "agrominerals" or "rock fertilizers", depending on the nature of the geological resource. These resources are sought after as inexpensive and local nutrient sources for crops. The low solubility of these materials compared to that of chemical fertilizers is thought to mitigate issues related to high nutrient mobility, like the runoff of excessive nutrients (Bakken et al. 1997a; van Straaten 2006). However, their low solubility also hinders widespread use of unprocessed rocks because their mineral components must be weathered before they can be accessible to plants (van Straaten 2006). Carbonatite rocks are particularly appealing as rock fertilizers, because the carbonate minerals that they contain have high weathering potential compared to silicate minerals (Chou et al. 1989).

The aim of this review is to assess the agronomic potential of carbonatite rock fertilizers. First, a systems perspective and overview of agrogeology will be covered. Second, the research that has been conducted on carbonatites used in agriculture will be reviewed. Third, the factors that affect mineral weathering will be briefly covered to inform appropriate assessment and utilization of carbonatites as nutrient resources. Finally, considerations will be presented to guide the further study of carbonatites or other similar rock fertilizers.

Sustainability and agricultural systems

One of the largest concerns in modern agriculture is sustainability. There are a number of issues that contribute to the challenge of achieving agricultural sustainability, such as the changing climate (Campbell et al. 2016; Altieri and Nicholls 2017), the growing global population (Alexandratos 2005), the continued use of non-renewable resources (e.g., phosphate rock resources; Cordell et al. 2009), and the depletion of nutrients, especially micronutrients, in agricultural systems (van Straaten 2007). Additionally, there are several prominent examples of how improperly managed agroecosystems can cause environmental damage, namely the degradation and erosion of farm soils

(Matson 1997; Tilman et al. 2002) and the runoff of highly-soluble nutrients and subsequent eutrophication of connected water bodies (Diaz and Rosenberg 2008; Savard et al. 2010).

In addressing issues related to sustainability, it is important to keep a systems-level view. A single agroecosystem will have a large number of components, from soil micro- and macro-fauna to economic and social factors, with an accompanying level of complexity. For simplicity, here the system is defined as plants, soils, and soil microorganisms as well as the interactions between them. The focus of agriculture is the production of plant biomass for consumption or industrial use (e.g., canola oil for biodiesel), and several factors contribute to proper plant growth (Figure 2.1a). Plants require six macro-nutrients (N, P, K, Ca, Mg, S), and depending on the plant, between seven (Fe, Mn, Zn, Cu, B, Cl, Mo) to twelve (Na, Co, Va, Ni, Si) micronutrients (Havlin et al. 1999). Additionally, proper growing conditions, such as temperature, soil pH, and soil moisture contribute strongly to plant success (Tilman et al. 2002). These requirements vary depending on the plant species. For the annual legume *Pisum sativum* L. (pea;), temperatures between 10 - 24 °C, 800 - 1200 mm of annual rainfall, very bright light conditions, and soil pH between 5.5 - 7 are recommended (FAO Ecocrop profile for pea). The annual cereal *Triticum aestivum* L. (wheat) has similar recommended soil pH and light conditions to those of pea, but prefers warmer temperatures between 15 - 23°C and a tighter water range of 750 to 900 mm annual rainfall (FAO Ecocrop profile for wheat). Mutualistic soil microbial partners, such as rhizobia for leguminous plants or arbuscular-mycorrhizal fungi for wheat and many other plants, are also important contributors to plant health. These mutualisms often allow plants to have improved access to nutrients (Lodwig et al. 2003; Carbonnel and Gutjahr 2014).

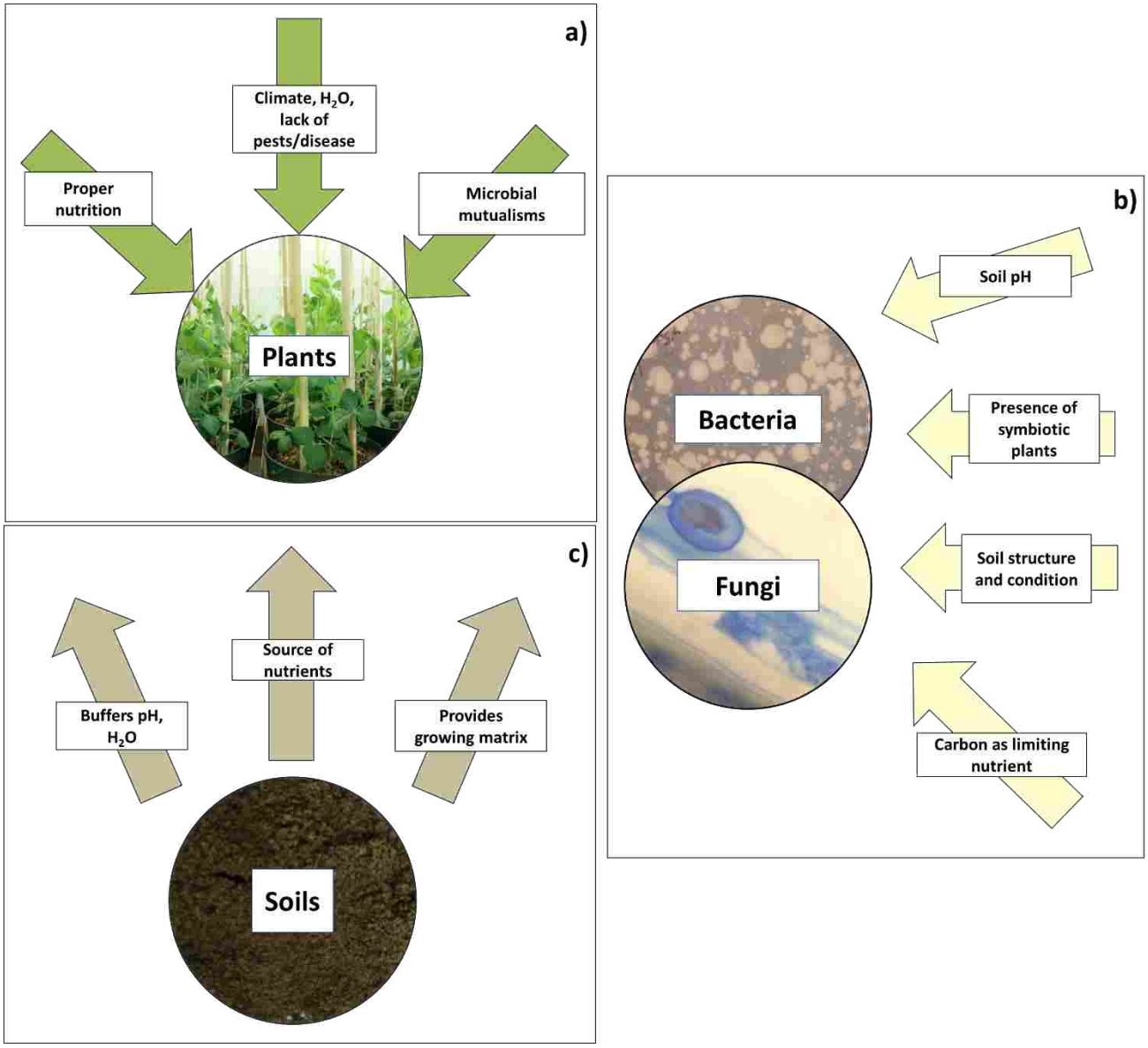


Figure 2.1: Components of a simple agroecosystem. a) For plants, appropriate nutrient levels, climatic conditions (e.g., temperature and photoperiod), moisture regime, minimization of pest and disease pressures, and the presence of microbial mutualist partners (e.g., mycorrhizal fungi) are all important factors for growth. b) The abundance and activity of soil microorganisms are strongly affected by the presence of plants as mutualistic partners or nutrient sources, by the stability of soil aggregates (e.g., relating to aeration, moisture content, and habitats for microbionts), by the acidity or alkalinity of the soil, and by the availability of nutrients (e.g., carbon is often limiting). c) Soils serve as a source of nutrients, as a buffer against water and pH changes through organic matter content, and as a growing matrix for plants and a habitat for microorganisms.

Microorganisms are important players in agroecosystems. Soil bacteria and fungi have certain growth requirements (Figure 2.1b) and are present in vast numbers (e.g., 10^8 cells per g of soil; Raynaud and Nunan 2014). Because of the large number of microorganisms in soils, it is difficult to assign specific functions (e.g., organic matter degradation) to specific microbial species, and microorganisms are often grouped by their functional roles. For instance, the response of nutrient-cycling microorganisms to liming has been measured through changes in the activity of soil enzymes (Acosta-Martínez and Tabatabai 2000). Often the limiting nutrient for soil microbial populations is carbon but in cases where nitrogen and phosphorus are limiting, the ratio between these three elements is important to consider (Demoling et al. 2007). Excesses of certain elements, like Cu or Mn, may inhibit microorganism survival depending on the species-specific tolerances to these elements (Gadd and Griffiths 1977). Certain *Arthrobacter*, *Bacillus*, and *Micrococcus spp*. are found on Mn-rich rocks, which implies a high-tolerance of these bacteria to that element (Hungate et al. 1987). Soil pH also influences microorganisms; most soil bacteria thrive at approximately neutral pH (Lauber et al. 2009) whereas fungi usually have broader pH tolerances (Rousk et al. 2010). However, these are generalizations: for instance, a *Glomus sp.* (WUM3) intended for use in agriculture was not tolerant of acidic conditions and had poor spore germination and hyphal growth when the soil pH was less than 5 (Porter et al. 1987). The amount of organic matter has also been shown to impact microbial populations, with greater organic matter content leading to greater microbial biomass and diversity (Zhong et al. 2010). Plant-microbial mutualisms are often viewed in agriculture through their benefits to plants, but the microbial partners also receive nutritional and/or ecological benefits from such symbioses. For example, when associated with the roots of legumes, nitrogen-fixing rhizobia within nodules gain access to large amounts of plant photosynthetic carbon (Hacin et al. 1997) and an environment within the plant where they can replicate before re-entering soils (Timmers et al. 2000). Mycorrhizal fungi associate with plant roots and exchange nutrients like P for photosynthetic carbon with many types of plants (Carbonnel and Gutjahr 2014). In the case of arbuscular-mycorrhizal fungi, fungal structures are developed both within the plant and within the soil environments (Friese and Allen 1991).

Both plants and microorganisms depend on the abiotic soil component, comprised in most cases of organic matter, rocks, and minerals which provide a number of benefits to agroecosystems (Figure 2.1c). Minerals are crystalline materials with a more or less fixed chemical structure, whereas rocks are larger aggregates of one or more minerals (Brady 1974). It is through the continuous gradual breakdown of these materials by biotic and abiotic action that soils are formed (Brady 1974). The ability of a given soil to support biological life and complete ecosystem functions is referred to as soil health (Doran and Zeiss 2000). Aggregate stability, organic matter, and nutrient content all contribute to soil health (Doran and Zeiss 2000), and are means by which soils buffer water levels and other properties which are important to biota. Soil compaction and drainage are also relevant factors. The continuous feedback between soils and their biota means that soil health is a dynamic process that can increase or decrease over time. Because of this feedback, rocks and minerals have a continuous influence on the biological interactions taking place within soils. In an agricultural context, the geological-biological interactions are perhaps most predominant in acidic soils, where low pH allows Al typically bound to clay minerals to become more mobile and phytotoxic to plants (Havlin et al. 1999). Soils formed with minerals rich in Ca or Mg (e.g., sedimentary limestones) are more alkaline, and these elements can be taken up by plants as nutrients (van Straaten 2007). High Ca and Mg levels also contribute to the cation-exchange capacity (CEC) of soils, which is the ability of a soil to retain cations and a measure of how well it can supply these cations to plants (Brady 1974). Less known is how minerals influence soil microbial communities; they often do so by acting as nutrient sources (Uroz et al. 2015) or by influencing soil chemistry (Havlin et al. 1999). Microorganisms have been shown to preferentially colonize minerals bearing nutrients over those that do not (Rogers and Bennett 2004), and this effect can be strong enough to influence microbial community composition (Whitman et al. 2018). More generally, the activity and abundance of soil bacteria are strongly affected by soil pH (Lauber et al. 2009), and a common result of using lime to make agricultural soil pH more neutral is an increase in microbial activity (Fuentes et al. 2006).

Because each of these components is simultaneously affected by and affects the others (Figure 2.2), a systems-level approach is needed to understand how changes in one component can

benefit or hinder another component. However, even with a three-component system (Figure 2.2), there are a large number of factors that can influence how each component interacts with another, and it is necessary to simplify as much as possible. Therefore, while we use a systems-level approach, we will focus only on a few of the factors that influence the behaviour of the plant-soil-microorganism system, and how the addition of carbonatite rock fertilizers can alter this three-component system.

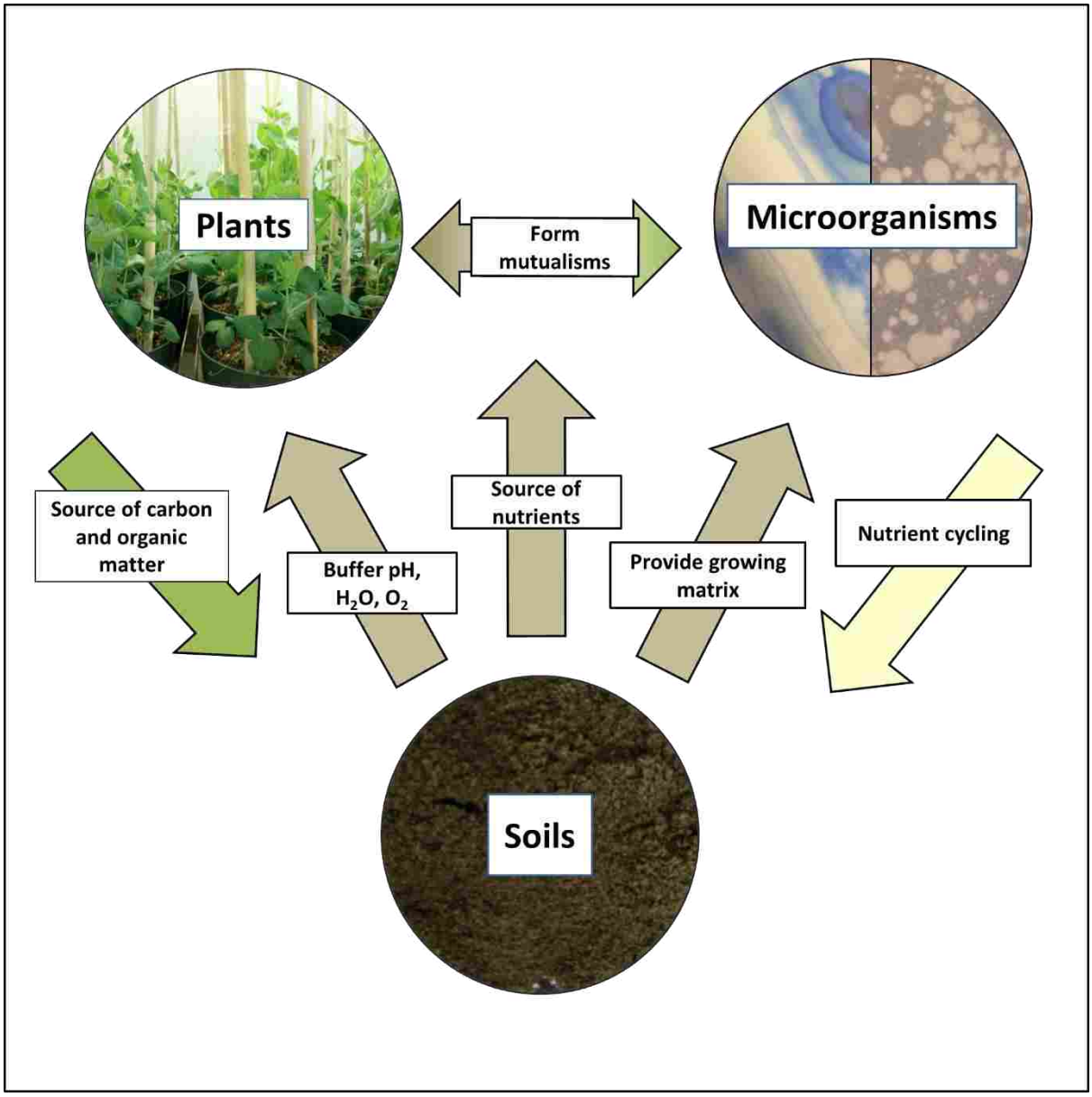


Figure 2.2: A few of the key interactions in a simple three-component agroecosystem. Because components interact with and are affected by other components, a systems-level view is necessary for understanding and interpreting changes, such as those expected with addition of carbonatite rock fertilizers. Arrows are colour-coded according to their component of origin.

Agrogeology, and the use of geology for agricultural benefit

Agrogeology as a field of study is directed at the use of geological resources to address problems in agriculture. Though using rocks or minerals in agriculture may be unusual to some, modern agriculture already strongly depends upon geological resources for soil amendments and as crop nutrient sources. At the field-level, application of calcitic and dolomitic limes is common practice to mitigate acidic (<5.5) soils and phytotoxic levels of elements like Al (Havlin et al. 1999). The dissolution of the lime depends strongly on the particle size (i.e., the reactive surface area) of the material and releases Ca^{+2} and/or Mg^{+2} into the soil solution (Havlin et al. 1999). The pH-neutralizing ability of lime is illustrated in the following chemical reaction (Havlin et al. 1999):

$$2\ Al^{+3}\ (\text{adsorbed}) + 3\ CaCO_3 + 3\ H_2O \rightarrow 3\ Ca^{+2}\ (\text{adsorbed}) + 2Al(OH)_3 + 3\ CO_2$$

The exchange of Ca^{+2} from lime with Al^{+3} adsorbed to soil particles leads to the formation of poorly-soluble aluminum hydroxides, and the added Ca^{+2} raises the soil pH by helping to balance the presence of H_3O^+ ions.

The use of rocks and minerals as nutrient sources has traditionally centred only on the plant component of an agroecosystem. The basis of most modern fertilizers is the reaction of phosphate rock (PR) or potassium-rich rock (e.g., potash) with chemicals to convert their nutrients into a highly water-soluble form (Zapata and Roy 2004; van Straaten 2007). The gains from this process depend strongly on the nutrient content of the rock, and low-value ores with less nutrients are not considered for conversion, as the cost to process the ore is higher than the expected profits. Those low-value ores are now being reconsidered as rock-fertilizers for sustainable agriculture (Zapata and Roy 2004). Much attention has been given to the use and efficacy of PRs as rock fertilizers, and they have been recognized by the UN Food and Agriculture Organization as important tools to achieve sustainable agriculture in developing countries (Zapata and Roy 2004). There is a wide variety of different PRs, but key examples are the various forms of apatite (mineral formula: $Ca_5(PO_4)_3(F,Cl,OH)$). Because rocks can be composed of one or more minerals, the term PR is often used liberally to mean any rock or mineral that contains P.

Other minerals, like nepheline ((Na,K)AlSiO4) or biotite ($K(Mg,Fe)_3AlSi_3O_{10}(F,OH)_2$), are also of interest in agrogeology, and are used as sources of K (Bakken et al. 1997a, 1997b; Zhang et al. 2018). Regardless of the specific nutrient in question, the limiting factor that determines the effectiveness of a given rock fertilizer or agromineral is its solubility. The solubility product constant (Ksp) for many minerals under abiotic conditions has been determined (e.g., carbonate minerals at standard temperatures and pressures; Chou et al. 1989) and is a function of the 3-D arrangement and bonding of the contained elements. While the laboratory Ksp values can be useful for predicting the efficacy of nutrient delivery to plants, the complexity of soils and agroecosystems often means the abiotic Ksp values do not effectively serve as a means of measuring mineral solubility under biotic conditions (Zhang et al. 2018). To counter this, many researchers have used plant nutrient content as a proxy measure for solubility or nutrient availability (e.g., Bakken et al. 1997a, 1997b; Burghelea et al. 2015; Myrvang et al. 2016). However, the inherently low solubility of most rocks and minerals is considered to limit their effectiveness as fertilizers (Harley and Gilkes 2000). Various methods have been proposed to counter this problem; one example is through the simultaneous application of P-minerals and P-solubilizing microorganisms in quantities appropriate to the specific agroecosystem (Reddy et al. 2002). The complexity of agroecosystems makes the usefulness of a particular method difficult to estimate, however, as plants, microorganisms, and plant-microorganism interactions are known to influence mineral weathering (Calvaruso et al. 2006; van Schöll et al. 2008; Uroz et al. 2009; Burghelea et al. 2015). One way around this complexity is to focus on rocks and minerals which weather rapidly. One group of rocks, the carbonatites, are considered to have high relative weathering rates compared to other rock types and this makes them appealing targets for agrogeological research.

Carbonatites are a form of igneous rock and are defined by their unusual composition: they have a majority of carbonate minerals and a minority of silicate minerals (Woolley and Kempe 1989). Many carbonatites are highly diverse in their mineralogical composition, and there are numerous minerals that have been found in association with carbonatite deposits (Table 1). Two noteworthy

examples of such accessory minerals are apatite and biotite (Woolley and Kempe 1989), already mentioned for their individual use as agrominerals.

Table 2.1: Non-exhaustive list of minerals organized alphabetically which have been found associated with carbonatite deposits. Only essential (e), common (c), and moderately common (m) minerals are included here following Heinrich (1980).

Name	Formula	Abundance	Mineral type
aegirine	$NaFeSi_2O_6$ (Fe as Fe^{3+})	c	silicates
albite	$NaAlSi_3O_8$	m	silicates
ankerite	$CaFe(CO_3)_2$	e	carbonate
antigorite	$(Mg,Fe)_3Si_2O_5(OH)_4$	m	silicates
apatite	$Ca_5(PO_4)_3(F,Cl,OH)$	c,e	phosphate
augite	$(Ca,Na)(Mg,Fe,Al,Ti)(Si,Al)_2O_6$	m	silicates
baddeleyite	ZrO_2	m/c	oxide/hydroxide
barite	$(Ba,Sr)SO_4$	c,e	sulfate
bastnaesite	$(Ce,La,Y)CO_3F$	m,e	oxide/hydroxide
betafite	$(Ca,U)_2(Ti,Nb,Ta)_2O_6(OH)$	m	oxide/hydroxide
calcite	$CaCO_3$	e	carbonate
chalcopyrite	$CuFeS_2$	m	sulfide
chlorite	$(Mg_5,Fe_5,Ni_5,Mn/Al)(AlSi_3)O_{10}(OH)_8$	m	silicates
chrysotile	$(Mg,Fe)_2SiO_4$	m	silicates
columbite	$(Fe,Mn)Nb_2O_6$	m	oxide/hydroxide
crocidolite	$Na_2(Fe^{+2}(_3),Fe^{+3}(_2))Si_8O_{22}(OH)_2$	m	silicates
diopside	$MgCaSi_2O_6$	m	silicates
dolomite	$CaMg(CO_3)_2$	e	carbonate
fersmite	$(Ca,Ce,Na)(Nb,Ta,Ti)_2(O,OH,F)_6$	m	oxide/hydroxide
fluorite	CaF_2	c/e	halide
galena	PbS	m	sulfide
hematite	Fe_2O_3	c/e	oxide/hydroxide
ilmenite	$FeTiO_3$	m/c	oxide/hydroxide
magnetite	Fe_3O_4	c/e	oxide/hydroxide
microcline	$KAlSi_3O_8$	m	silicates
monazite	$(Ce,La,Nd,Th,Sm,Gd)PO_4$	m	phosphate
nepheline	$Na_3KAl_4Si_4O_{16}$	m	silicates
olivine	$(Mg_{+2},Fe_{+2})_2SiO_4$	m	silicates
orthoclase	$KAlSi_3O_8$	m	silicates
perovskite	$CaTiO_3$	m/c	oxide/hydroxide

Table 1: continued			
Name	**Formula**	**Abundance**	**Mineral type**
phlogopite	$KMg_3AlSi_3O_{10}(F,OH)_2$	m	silicates
pyrite	FeS_2	c	sulfide
pyrochlore	$(Na,Ca)_2Nb_2O_6(OH,F)$	c/e	Oxide/hydroxide
pyrrhotite	Fe(1-x)S, x=0-0.2	m	sulfide
quartz	SiO_4	c	silicates
rutile	TiO_2	m/c	Oxide/hydroxide
siderite	$FeCO_3$	m/e	carbonate
sphalerite	Zn/FeS	m	sulfide
sphene	$CaSiO_5$	m	silicates
strontianite	$SrCO_3$	m	carbonate
vermiculite	$(Mg, Fe^{+2},Fe^{+3})_3(Al,Si)_4O_{10}(OH)_2*4H_2O$	m	silicates
vesuvianite	$Ca_{10}(Mg,Fe)_2Al_4(SiO_4)_5(Si_2O_7)_2(OH,F)_4$	m	silicates
wollastonite	$CaSiO_3$	m	silicates
zircon	$ZrSiO_4$	m	silicates

Worldwide, there are 527 identified carbonatite deposits, of which 477 have been characterized in terms of their mineralogy (Woolley and Kjarsgaard 2008a, 2008b). The majority of described deposits are associated with currently-stable tectonic fold zones (Jones et al. 2013), and can be found in Africa (32% of known deposits), Asia (30%) and North America/Greenland (21%; Woolley and Kjarsgaard 2008a). As other geological resources (e.g., PR rocks), carbonatites are **non-renewable** - currently the only active carbonatite volcano is the Ol Doinyo Lengai natrocarbonatite volcano in Tanzania (Vaughan et al. 2008; Jones et al. 2013) and many deposits are estimated to be millions or billions of years old (Woolley and Kjarsgaard 2008b). Owing to their diverse mineral compositions, carbonatites have been named according to their primary carbonate mineral (e.g., calciocarbonatite; Woolley and Kempe 1989) or by the type of silicate rock they are associated with (e.g., carbonatite with nephelinite; Woolley and Kjarsgaard 2008a). For agricultural purposes, the two most relevant carbonate types, according to their primary carbonate mineralogy, are calciocarbonatites (Ca-rich) and magnesiocarbonatites (Mg-rich). Other classes of carbonatites exist, for example ferrocarbonatites (Fe-rich), but these will not be touched on in this review as they are less relevant to agriculture and are uncommon.

Despite their appeal as agricultural materials, carbonatites have only been minimally explored for their agronomic potential, as it has been thought that their high Ca and Mg contents limit their ability to effectively release nutrients (van Straaten 2002). Indeed, works on rock fertilizers and agrominerals have been focused on silicate rocks and either briefly mention carbonatites (e.g., Gough and Herring 1993, van Straaten 2007) or do not mention them at all (Zhang et al. 2018). Also, studies of carbonatites (and often rock fertilizers in general) center around applied research aspects (i.e., "does it work?") and not basic research aspects (i.e., "how does it work"). Despite the limited research, carbonatites have demonstrated effectiveness in serving as nutrient sources for plants (Bakken et al. 1997b, 1997a; Jones 2016; Myrvang et al. 2016). They can also be effective as liming agents (e.g., Myrvang et al. 2017), as calciocarbonatites and magnesiocarbonatites have high contents of igneous calcite and dolomite (Woolley and Kempe 1989). It can also be difficult to identify whether a given study is on carbonatites or not because of the inconsistent or unclear usage of geological terms (e.g., is it a phosphate mineral, or a rock which contains phosphate minerals, or a phosphate rock?). A specific carbonatite can thus be reduced to simply a "phosphate rock" which neglects reference to and integration of the complex mineralogy of these rocks. For instance, Spanish River Carbonatite (SRC) is referred to in Arcand et al. (2010) as a phosphate rock "…originating from a carbonatite deposit…". Although apatite is a major constituent of SRC, it has been estimated to also contain around 30 other minerals (Sage 1987). In this review, I will discuss only those studies that specifically refer to using carbonatites.

The agronomic use of carbonatites

Among the agricultural carbonatites, the Lillebukt Alkaline Complex carbonatite at Stjernøy, Norway, has received particular attention. Bakken et al. (1997a) conducted an early study comparing plant yield and K content of Italian ryegrass (*Lolium multiflorum italicum var*. Torilo) growing in two soils (pea, loamy sand or silt loam) amended with several rocks and minerals from, including carbonatite from Lillebukt. In all soils, the carbonatite was able to provide similar amounts of K to the plants as KCl, and carbonatite-treated plants had roughly 0.8% more K content in their dry matter than the plants grown with other rocks from the mine tailings (Bakken et al. 1997a). Along with plants

given KCl, plants provided with carbonatite also had significantly higher dry weight yields than plants in the other treatments. Bakken et al. (1997b) tested the efficacy of several K-containing rocks as slow release K-fertilizers for barley (*Hordeum vulgare* L.) grown in peat moss, and included two carbonatite rocks from the Lillebukt complex with different associated minerals (one with nepheline and one with biotite). There, the carbonatite varieties differed in their ability to provide K, where approximately 50% of the K provided as biotite-carbonatite was taken up by the plants and less than 25% was taken up from the nepheline-carbonatite (Bakken et al. 1997b). Plants given biotite-carbonatite also yielded nearly as much total plant dry matter as those plants provided with KCl: 112 g pot^{-1} for biotite carbonatite, 90 g pot^{-1} for nepheline carbonatite, and 137 g pot^{-1} for KCl. The authors concluded that the carbonatites might act too rapidly to serve as slow-release fertilizers (Bakken et al. 1997b). These studies indicate that carbonatites are effective nutrient sources for plants and can be similar in effectiveness to water-soluble chemical fertilizers. More recently, the Lillebukt deposit was assessed for the nutrient content of natural vegetation overlying the deposit and to determine whether the plants were negatively affected by the presence of potentially phytotoxic levels of Ba (Myrvang et al. 2016). Barium is of particular concern because elevated levels in plants are known to depress photosynthesis and plant growth (Suwa et al. 2008). The authors reported that the surveyed herbs, dwarf-shrubs, and grasses growing above the complex had higher levels of K, Mg, P, Ca, Sr, and Ba in their tissues than would be expected from literature values, and that the Ba did not appear to negatively affect the plants. Plants which were growing above carbonatite rocks were compared to plants growing above silicate rocks (apatite-pyroxenite-hornblende), but similarities in acid-extractable nutrient concentrations between the sites made it difficult to use the plants above the silicate rocks as a control group. Despite some remaining concerns over the potential phytotoxicity of Ba from the Lillebukt deposit, the carbonatite there has demonstrated capacity for nutrient release and plant uptake, and may also be useful as agricultural lime (Myrvang et al. 2017). While these studies provide invaluable information regarding the efficacy of carbonatite as nutrient sources and some of their effects on soils, it is still unclear how carbonatites affect agroecosystems as a whole. For instance, the microorganism component of those systems was not explored.

The changes induced on the ecosystem overlying the Spanish River Carbonatite (SRC) deposit were recently explored by Jones et al. (2019; **Chapter 3**). There, soil samples were segregated into influence-categories based on differences in soil chemistry. Soils influenced by SRC had higher soil pH, higher CEC, and higher Ca/Mg/Mn levels than soils with low or negligible carbonatite influence. Broadly speaking, SRC appears to have led to the creation of 'islands' of more basic soil (~pH 6) in an otherwise acidic (~pH 5) forest soil (Jones et al. 2019). The changes in soil chemistry were found to drive changes in microbial abundance and plant community composition. In terms of microorganisms, the abundance of several bacterial OTUs were decreased in highly SRC-influenced soils compared to soils with low/negligible influence. However, two OTUs from the Galliaceae and Micrococcaceae family were of higher abundance. Fungi were less affected by SRC than bacteria, though there was likely an interaction between fungal abundance and tree species. Specifically, many of the differentially-abundant fungal OTUs were identified as mycorrhizae, and tree species (i.e., mutualistic hosts) were typically dissimilar between sites. The herb and shrub communities in SRC-influenced soils were similar to those located on soils not influenced or outside the deposit, but comprised more ruderal plant species. This study, while not in an agricultural setting, can inform what field-level agroecosystem responses might be expected from carbonatite addition.

A preliminary investigation of SRC impacts on pea growth (*Pisum sativum* L.) and nodulation was undertaken by Jones (2016). Under controlled conditions in an inorganic mixture consisting of a 1:1 mix of vermiculite:Turface™, the effects of various concentrations of SRC were tested across seedling, vegetative, and reproductive growth stages. The usefulness of the nitrogen-fixing symbiosis, as estimated by three parameters derived from nodule and host dry weights, was also assessed. Although only minor changes in root growth were observed at a ratio of 1:10 SRC:substrate over plants in unamended substrates, dramatic changes in nodulation were detected. When grown with SRC, nodulated plants developed nearly twice as many nodules as, and had more beneficial nodulation than, those grown in unamended substrate. However, it was unclear whether these benefits were specific to SRC or a function of increased substrate pH changes. Despite this, it was demonstrated that pea could be grown in substrates with SRC as their exclusive nutrient source

provided that plants were nodulating and therefore had access to nitrogen (Jones 2016). The uncertainty around the beneficial effects of SRC on pea was addressed in a follow-up study (**Chapter 4**). There, the vermiculite:Turface™ mix was again used and the substrate pH was controlled for by adding calcitic lime. It was found that the aforementioned benefits to nodulation are likely the result of pH changes, as pea amended with calcitic lime had similar nodulation characteristics to those of pea amended with SRC (**Chapter 4**). Also, the use of a simplified systems-level view was enlightening, as the growth responses of pea to carbonatite addition changed when plants were inoculated with agricultural microorganisms (**Chapter 4**). This indicates that interactions between plants-microorganisms-carbonatite need to be considered when assessing carbonatite effectiveness.

In the same study (**Chapter 4**), the effects of SRC on wheat (*Triticum aestivum* L.) were also investigated, and compared against the effects of calcitic lime when plants were grown in vermiculite:Turface™. Notably, wheat showed strong positive responses when grown in carbonatite-amended soils compared to plants grown in lime-amended soils, and this was determined to be predominantly driven by nutrient acquisition from the carbonatite. Unlike lime, SRC did not increase the microbial activity of the substrates (measured by substrate CO_2 exudation) compared to control substrates. Since the response of wheat to SRC was predominantly nutrient-based, the extent to which microorganisms influence the response of wheat to carbonatite remains to be answered. It is also still unclear why SRC did not increase microbial activity in the same way as lime, as both minerals were expected to function similarly in this regard. The presence of elements in SRC which are not in lime, such as Mn, may be responsible.

The interplay between SRC, plants, and substrate microorganisms was also explored by Christie (2019), who looked primarily at microbial responses to combinations of cover crops and soil amendments (including SRC) under greenhouse conditions. There, two different cover crop combinations of one legume and one forb (alfalfa and chicory or red clover and oilseed radish) and three soil amendments (chemical nitrogen, chemical fertilizer, or SRC) were tested for their effects on the soil microbial respiration and number of culturable heterotrophic, phosphate-solubilizing or nitrogen-fixing bacteria. While SRC showed an overall positive effect on most parameters, increasing

the number of culturable bacteria and the value of nodulation to legumes, the cover crop combination also influenced the results. As an example, the number of phosphate-solubilizing bacteria was the highest in the chemical fertilizer treatment with alfalfa and chicory, but was the highest with the SRC treatment with red clover and oilseed radish (Christie 2019). Similarly, soil microbial respiration was the lowest of the three treatments when SRC was provided to the soils of alfalfa and chicory, but was the highest when SRC was provided to the soils of red clover and oilseed radish. While the soil and nutrient conditions in this study were different from those of previous studies with SRC (Jones 2016; **Chapter 4**), it further highlights the importance of taking a system-level view when testing the efficacy of carbonatites as rock fertilizers. Interactions between plants-microorganisms-carbonatites can give contrasting results (e.g., with soil microbial respiration), and neglect of these interactions can lead to incorrect conclusions or misleading results.

In a parallel study to Christie (2019), similar amendments and cover crop combinations (alfalfa and chicory, red clover and oilseed radish, or annual ryegrass alone) were tested in an operational vineyard in Ontario, Canada (VanVolkenburg 2019). Under the first year of testing under field conditions, there was no measured effect of the amendments, including SRC, on the plant and soil macroinvertebrate community diversity, though the choice of cover crop was important. For example, a ryegrass (*Festuca perennis* Lam.) cover crop increased the plant community diversity over cover crop mixtures but did so inconsistently, while the forb and legume cover crop mixtures gave a more even distribution of plant species in the test areas (VanVolkenburg 2019). There was also a strong effect of season on the plant and soil faunal diversity, and the author emphasized that multi-year studies are a necessity to fully understand agroecosystem responses to carbonatite addition.

In a field study, Arcand et al. (2010) explored the use of phosphate rocks, including SRC, in their capacity for delivering P to buckwheat (*Fagopyrum esculentum* Moench). The intent was to utilize buckwheat, a plant that acidifies its rhizosphere, to obtain P from the various PRs, and then apply the buckwheat crop residues as a P source for organic agriculture. It was expected that the acidic rhizosphere of buckwheat would help to solubilize P from the phosphate rock and increase plant P uptake, and then the P would be more accessible to future crops in the form of organic matter

than in the form of a PR. However, the results did not indicate good P uptake from the carbonatite, and since the test soil was found to have high Ca content and more basic pH, the authors attributed their findings to the soil conditions limiting carbonatite weathering. This study further emphasizes that the context in which carbonatites are used must be considered for their effectiveness to be realized. Because of the high amounts of Ca and Mg in basic soils, carbonatites are unlikely to be effective as rock fertilizers as these would limit mineral breakdown and nutrient release (as in Arcand et al. 2010). In acidic soils, rock weathering is promoted and carbonatites should be effective as both nutrient-sources and liming agents (as in (Bakken et al. 1997a, 1997b; Myrvang et al. 2017).

Aside from these studies, work on carbonatites is erratic and sparse. Briefly, carbonatites are covered in a review of geological resources for sustainable agriculture (Gough and Herring 1993), but they are lumped together and considered like PR. There is also interest in exploiting carbonatites in the People's Republic of China, but this is largely in terms of non-agricultural resources (e.g., as a source of rare earth elements for electronics manufacturing; Xu et al. 2010). However, there is evidence to suggest that rare earth elements can promote plant growth (e.g., by acting analogously to Ca^{+2}, Hu et al. 2004; Tyler 2004), and so industrial development of carbonatite deposits may open them up to agricultural usage in the future.

In many of the studies of agricultural carbonatites, these rocks are treated like simple nutrient sources for plants, and have often been considered analogous to PR. However, the recent research with SRC has demonstrated that the mineralogical complexity of carbonatites and the interactions occurring in agroecosystems must be considered for proper interpretation of research findings. The microorganisms-carbonatite interactions especially have only been minimally studied yet seem to play an important role in modulating crop responses to carbonatite (e.g., as seen with pea). A detailed understanding of how carbonatites can affect and are affected by different agroecosystem components is required for their effective use. This knowledge is still lacking. Anecdotal studies on carbonatites conducted by farmers (e.g., a comparison between SRC, wollastonite, and basalt for effects on carrot flavour and nutrition; https://bluegrassfarm.ca/pass-the-basalt/) indicate the desire and need for this knowledge.

Mineral weathering, biological action, and nutrient release

The weathering of minerals is the result of the cumulative action of abiotic and biotic components in an agroecosystem (Figure 2.3). While incubation experiments under various conditions and analysis of the resulting soluble elements give a good indication of the weathering potential of a given mineral (e.g., Chou et al. 1989), under natural or field conditions it is harder to measure weathering. In studies on rock fertilizers and agrominerals, the differences in the nutrient content between plants grown with or without the minerals are often used as a measure to indicate nutrient release (Bakken et al. 1997a, 1997b; Arcand et al. 2010; Myrvang et al. 2016). In general, important abiotic determinants are the composition, the surface area and texture of a mineral, and the environmental conditions to which it is subjected to, like temperature, water content, and pH (Goldich 1938; Harley and Gilkes 2000). The amount of silicate in a given rock or mineral has also been considered indicative of its resistance to weathering (Goldich 1938; Harley and Gilkes 2000). Geological materials with fewer silicates (e.g., calcite) are expected to weather more rapidly than those with more silicates (e.g., quartz). Carbonatite rocks are formed predominantly of carbonate minerals - under acidic pH and abiotic sterile laboratory conditions, carbonate mineral dissolution depends on the movement of dissolved ions away from the mineral surface, on the ambient temperature, and on the concentration of CO_2 in solution (Chou et al. 1989). These factors are expected to be drivers of carbonatite weathering with agricultural use. However, carbonatites are not usually in abiotic environments, and in agriculture they will be subjected to a variety of biotic pressures that can affect their breakdown.

One of the major biotic components that minerals encounter are microorganisms, and factors that affect microorganisms can influence the extent to which they do or do not weather minerals (Figure 2.3). Directly, bacteria (Rogers and Bennett 2004; Whitman et al. 2018) and fungi (Whitman et al. 2018) both actively take up dissolved nutrient ions from weathering minerals, and produce a variety of compounds such as low-molecular-weight organic acids and siderophores to increase mineral dissolution (van Schöll et al. 2008; Ahmed and Holmström 2015). Indirectly, factors such as the presence of a preferred carbon substrate (glucose being a specific example for mineral-weathering

bacteria; Hameeda et al. 2006; Uroz et al. 2007), appropriate soil pH (e.g., neutral pH for bacteria; Lauber et al. 2009; Rousk et al. 2010), and the presence of mutualistic hosts (e.g., for fungi; van Schöll et al. 2008; Burghelea et al. 2015) can modulate mineral weathering by affecting the activity of microorganisms at the mineral surface. Microorganisms are thus expected to play a key role in weathering carbonatites added to soils. The "mineralosphere" is a term coined by Uroz et al. (2015), who view soil-based minerals as inorganic analogues to plant roots in terms of their effects on microbial population growth. It has recently been demonstrated that the type and composition of minerals influence the microbial communities which develop around them in a manner similar to roots (Whitman et al. 2018). This builds on previous work by Colin et al. (2017), where both the mineral type and tree species were found to influence the soil microbial communities. Although the number of microbial types on the minerals was comparable to that of the bulk soil, both communities were composed of different members. By growing in the mineralosphere, microorganisms gain access to nutrients like iron (Rogers and Bennett 2004; Whitman et al. 2018), manganese (Colin et al. 2017), phosphorus (Bennett et al. 2001; Rogers and Bennett 2004) and trace nutrients like silicon (Ferris and Lowson 1997). Banfield et al. (1999) proposed that autotrophic carbon from plants helps fuel microbial weathering of nutrient-bearing minerals; their model is based on the interaction between minerals and lichens. However, the autotrophic carbon does not need to come from plants. A study by Ferris and Lowson (1997) found that in the sedimentary dolomitic limestone of the Niagara escarpment, a microbial ecosystem had developed around mineral weathering. Cyanobacteria and other microbes were thought to act in producer roles, with their fixed carbon being utilized by heterotrophic microorganisms to acquire trace nutrients from the dissolution of the rock. The ability of microorganisms to increase weathering of nutrient-bearing minerals has also been the subject of basic research aimed at increasing the effectiveness of these minerals to deliver nutrients for plants. For instance, apatite was demonstrated to be solubilized by various *Aspergillus tubingensis* and *A. niger* strains (Reddy et al. 2002), and by *Bacillus*, *Rhizobium*, and *Pseudomonas* (Arcand and Schneider 2006). Prior to application, minerals would be incubated with the microorganisms to help increase mineral weathering and reactivity (Arcand and Schneider 2006).

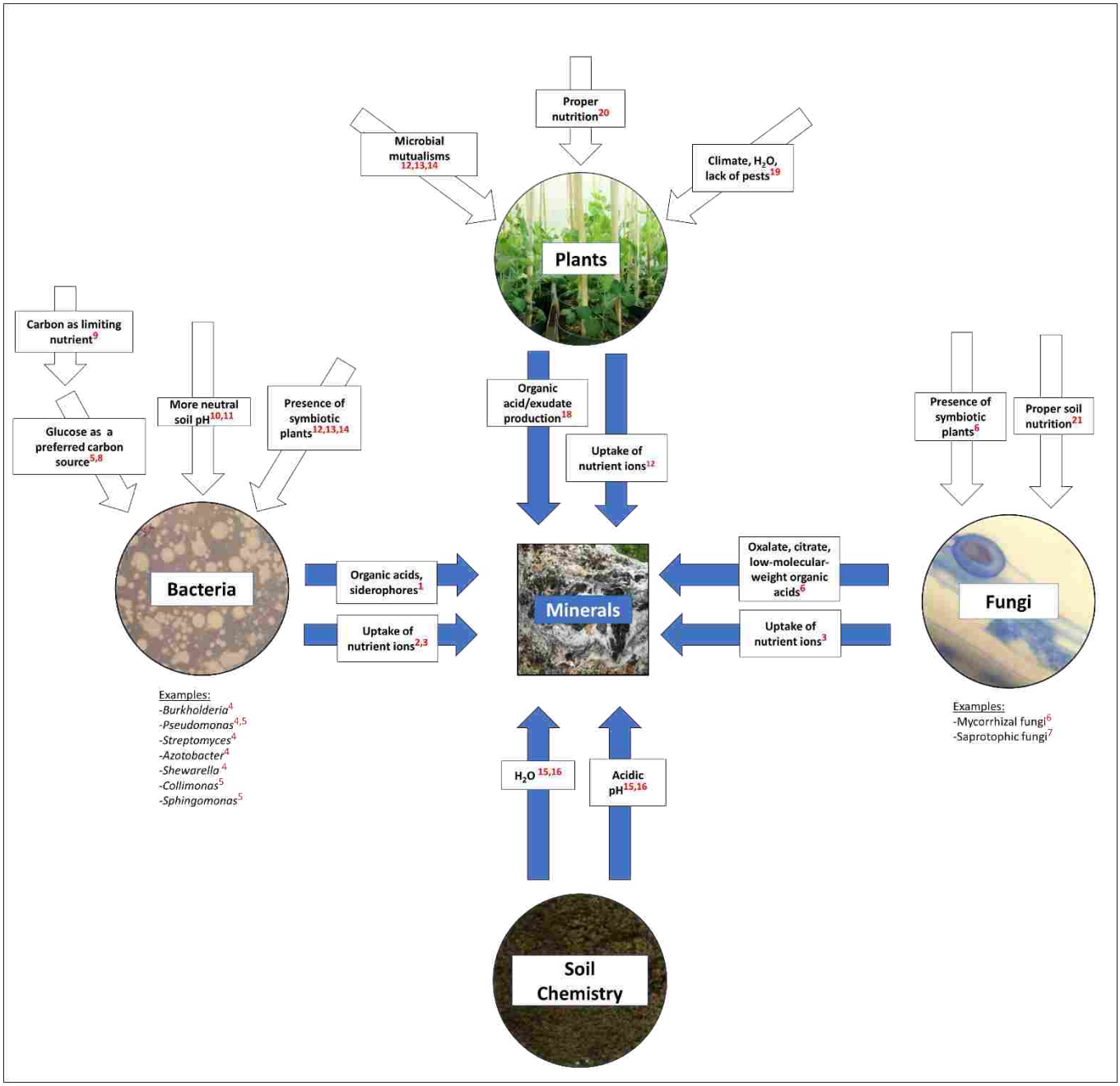


Figure 2.3: Key direct (blue arrows) and indirect (white arrows) factors influencing mineral weathering under agroecosystem conditions. Direct factors are those that act at the level of the mineral itself to promote dissolution (e.g., acidic pH). Indirect factors are higher-level effects that impact the activity of biological components which then increases the direct effect of these components on mineral dissolution. When investigating the use of carbonatites or other rock fertilizers, care should be taken to consider both indirect and direct effects.

References: **1** – Ahmed and Holmström (2015) Chem. Geol. 403:13-23; **2** – Rogers and Bennet (2004) Chem. Geol. 203:91-108; **3** – Whitman et al. (2018) Environ. Microbiol. 20: 4444-4460; **4** – Uroz et al. (2009) Trends Microbiol. 17: 378-387; **5** – Uroz et al. (2007) Appl. Environ. Microbiol. 73: 3019-3027; **6** – van Schöll et al. (2008) Plant Soil 303: 35-47; **7** – Ceci et al. (2018) Ambio 47(S1): 30-40; **8** –Hameeda et al. (2006) Curr. Microbiol 53: 298-302; **9** – Demoling et al. (2007) Soil Biol. Biochem. 39: 2485-2495; **10** – Lauber et al. (2009) Appl. Environ. Microbiol. 75: 5111-5120;

11 – Rousk et al. (2010) ISME J. 4:1340-1351; **12** – Calvaruso et al. (2006) Appl. Environ. Microbiol. 72: 1258-1266; **13** – Burghelea et al. (2015) Biogeochemistry 124: 187-203; **14** – Phillipot et al. (2013) Nat. Rev. Microbiol. 11: 789-799; **15** – Harley and Gilkes, (2000) Nutr. Cycl. Agroecosys. 56: 11-36; **16** – Goldich (1938) J. Geol. 46: 17-58; **17** – Chou et al. (1989) Chem. Geol. 78: 269-282; **18** – Badri and Vivanco (2009) Plant Cell Environ. 32: 666-681; **19** – Tilman et al. (2002) Nature 418: 671-677; **20** – Havlin et al. (1999) ISBN 10: 0136268064; **21** – Zhong et al. (2010) Plant Soil 326: 511-522.

Another major biotic component that minerals encounter are plants. Like microorganisms, plants are also considered in terms of their indirect and direct actions toward mineral weathering (Figure 2.3). Directly, they can increase weathering by exuding organic acids (e.g., isocitric acid; Arcand and Schneider 2006; Badri and Vivanco 2009), by taking up newly-liberated ions from the nearby mineral solution, and through beneficial interactions with rhizospheric microorganisms (e.g., mycorrhizal fungi; Burghelea et al. 2015). Indirect factors that affect plant growth are also expected to alter the ability of plants to weather minerals, like increased growth benefits from microbial mutualisms (Calvaruso et al. 2006; Philippot et al. 2013; Burghelea et al. 2015), proper plant nutrition (Havlin et al. 1999), growing conditions and pest pressures (Tilman et al. 2002). The intersection between rhizosphere and mineralosphere (i.e., the interplay between microorganisms and plant roots) seems especially important to mineral weathering and effective rock fertilizer use. For example, significant increases in the weathering of biotite due to the action both of pine roots (*Pinus sylvestris*) and strains of *Burkholderia glathei* were found by Calvaruso et al. (2006) through direct observation of the minerals via scanning-electron microscopy. A bacterial endophyte (*Enterobacter asburiae* strain 3Fll) that colonizes root hairs of *Zea* sp. and *Lolium multiflorum* was noted to increase the length of root hairs but also to help solubilize rock P through rhizosphere acidification (Shehata et al. 2017). With longer root hairs, the plants had a higher absorptive surface area to take up solubilized P from the bacteria. Because of these and other examples, it is expected that the actions of both microorganisms and plants will act synergistically to promote weathering of rock fertilizers and agrominerals in a manner similar to that proposed by Banfield et al. (1999). Much additional work is needed to characterize these interactions, however.

Ultimately, both the abiotic and biotic components of mineral weathering need to be considered when evaluating rock fertilizers in order to maximize their effectiveness. Broad predictions can be made as to the impacts and effectiveness of carbonatite rock fertilizers in a simple three-component system (Figure 2.3), but the complexity of natural agroecosystems means a detailed understanding of the interactions between components is needed to inform appropriate usage of carbonatites. What works well in one agroecosystem may not be successful for another. For instance, rock fertilizers are not expected to be effective in basic soils because mineral dissolution is not favoured.

Estimating the usefulness of carbonatites

Research on agricultural carbonatites has covered only a handful of the known global deposits. Because of the mineralogical diversity of carbonatites (Table 1), a means of predicting whether a given carbonatite will be agronomically useful would be beneficial. We propose three properties which can be used as a starting point to assess the potential of a carbonatite deposit using available geological information:

1) The nutrient mineralogy of the deposit.

2) The quantity of the material contained within the deposit.

3) The deposit's location relative to the intended sites of use and to ecologically-sensitive area(s)

The nutrient mineralogy of a deposit is a key consideration for determining the potential usefulness of a carbonatite and the agricultural contexts which it might be useful in. As examples, two deposits that show promising nutrient composition are the Lillebukt carbonatite deposit in Norway (Myrvang et al. 2017) and the SRC deposit in Canada (Sage 1987; Jones et al. 2019). Both deposits are composed of a calcite carbonatite with accessory biotite and apatite minerals, which would serve as sources of calcium, potassium, and phosphorus, respectively. Conversely, the presence of harmful elements like uranium may prevent exploration, as is found in the Manitou Islands in Lake Nipissing

in Canada (Woolley and Kjarsgaard 2008b). Other elements, such as Ba or Mn, merit caution if present in carbonatites because improper use (e.g., used excessively in hypoxic soils) may lead to phytotoxicity. This is the case with Ba in the Lillebukt (Myrvang et al. 2016, 2017) and Mn in the Spanish River (Jones et al. 2019; **Chapter 4**) deposits.

Similarly, the volume of material within the deposit should be considered prior to exploiting a deposit. Carbonatites are non-renewable resources and the amount of carbonatite present is directly proportional to the economic viability of the deposit. The ore grade (amount of mineral in a specific ore) is also a factor, as deposits can be heterogenous in their mineralogy. For instance, the SRC deposit has three zones each with differing amounts of apatite, calcite, biotite, and vermiculite (Sage 1987; Jones et al. 2019). Finally, the location of the deposit is of prime importance as this directly relates to both the environmental impact of mining operations and the operational cost of mining and transporting the harvested material. The Elchuru carbonatite deposit in India and the Bull's Run deposit in South Africa (Woolley and Kjarsgaard 2008b) are near populated areas and share broad mineralogical similarity to the Spanish River and Lillebukt carbonatites, and so could be viable deposits to be developed. Deposits in ecologically-sensitive areas should not be considered. Because carbonatites are globally located, preference should be given to those deposits which are local instead of those geographically distant.

Ultimately, the effectiveness of carbonatites to serve a useful function in agriculture needs to be demonstrated with field trials under different conditions and with different crops. The appeal of carbonatites is that they can serve as locally-sourced, low-cost, and environmentally-friendly nutrient sources for crop plants and so they will be assessed according to how well they meet these criteria. However, given the complexities of mineral weathering (Figure 2.3), interactions of the carbonatite with plants, soils and soil microorganisms need to be considered during assessments. Also, carbonatites have not yet been assessed in terms of their potential to reduce or contribute to nutrient leaching/runoff (Myrvang et al. 2017).

Conclusions and future directions

Because of their high relative weathering rates compared to other rocks and their associations with nutrient-bearing minerals (e.g., apatite and biotite), carbonatites have strong potential as rock fertilizers for crop plant nutrition. They have a demonstrated capacity to serve as nutrient sources for plants, but research is limited in both substance (few carbonatites assessed) and scope (typically only plant biomass effects measured). Furthermore, the effect of carbonatites on plants depends strongly on context; i.e., the soil conditions, microbial communities, plant species, and time span in which they are used. Considerations beyond just the effects of carbonatites on plants are thus a necessity in understanding how plants-microorganisms-carbonatites-soils interact. This is highlighted by the complexities of mineral weathering, which can be affected by numerous factors even in a simple system. Additional work is also needed to further characterize which deposits can be considered agriculturally-relevant, and the impacts of carbonatites under suitable agricultural conditions. The environmental impacts of carbonatite use also need to be incorporated, whether in terms of benefits (e.g., less processing required for use as a fertilizer) or costs (e.g., development of deposit, removal of overlying biota, and environmental impact of the mining).

Literature cited:

Acosta-Martínez, V., and Tabatabai, M.A. 2000. Enzyme activities in a limed agricultural soil. Biol. Fertil. Soils **31**(1): 85–91. doi:10.1007/s003740050628.

Ahmed, E., and Holmström, S.J.M. 2015. Microbe–mineral interactions: The impact of surface attachment on mineral weathering and element selectivity by microorganisms. Chem. Geol. **403**: 13–23. doi:10.1016/j.chemgeo.2015.03.009.

Alexandratos, N. 2005. Countries with rapid population growth and resource constraints: Issues of food, agriculture, and development. Popul. Dev. Rev. **31**(2): 237–258. doi:10.1111/j.1728-4457.2005.00064.x.

Altieri, M.A., and Nicholls, C.I. 2017. The adaptation and mitigation potential of traditional agriculture in a changing climate. Clim. Change **140**: 33–45. doi:10.1007/s10584-013-0909-y.

Arcand, M.M., Lynch, D.H., Voroney, R.P., and van Straaten, P. 2010. Residues from a buckwheat (*Fagopyrum esculentum*) green manure crop grown with phosphate rock influence bioavailability of soil phosphorus. Can. J. Soil Sci. **90**(2): 257–266. doi:10.4141/cjss09023.

Arcand, M.M., and Schneider, K.D. 2006. Plant- and microbial-based mechanisms to improve the agronomic effectiveness of phosphate rock : a review. An. Acad. Bras. Cienc. **78**: 791–807.

doi:10.1590/S0001-37652006000400013.

Badri, D. V, and Vivanco, J.M. 2009. Regulation and function of root exudates. Plant, Cell Environ. (32): 666–681. doi:10.1111/j.1365-3040.2009.01926.x.

Bakken, A.K., Gautneb, H., and Myhr, K. 1997a. The potential of crushed rocks and mine tailings as slow-releasing K fertilizers assessed by intensive cropping with Italian ryegrass in different soil types. Nutr. Cycl. Agroecosystems **47**: 41–48. doi:10.1007/BF01985717.

Bakken, A.K., Gautneb, H., and Myhr, K. 1997b. Plant available potassium in rocks and mine tailings with biotite, nepheline and K-feldspar as K-bearing minerals. Acta Agric. Scand. Sect. B - Soil Plant Sci. **47**(3): 129–134. doi:10.1080/09064719709362452.

Banfield, J., Barker, W., Welch, S., and Taunton, A. 1999. Biological impact on mineral dissolution : Application of the lichen model to understanding mineral weathering in the rhizosphere. Proc. Natl. Acad. Sci. **96**: 3404–3411. doi:10.1073/pnas.96.7.3404.

Bennett, P.C., Rogers, J.R., Choi, W.J., and Hiebert, F.K. 2001. Silicates, silicate weathering, and microbial ecology. Geomicrobiol. J. **18**(1): 3–19. doi:10.1080/01490450151079734.

Brady, N. 1974. The nature and properties of soils, 5th edition. Macmillan Publishing Co., New York.

Burghelea, C., Zaharescu, D.G., Dontsova, K., Maier, R., Huxman, T., and Chorover, J. 2015. Mineral nutrient mobilization by plants from rock : influence of rock type and arbuscular mycorrhiza. Biogeochemistry **124**: 187–203. doi:10.1007/s10533-015-0092-5.

Calvaruso, C., Turpault, M.-P., and Frey-Klett, P. 2006. Root-associated bacteria contribute to mineral weathering and to mineral nutrition in trees: a budgeting analysis. Appl. Environ. Microbiol. **72**(2): 1258–1266. doi:10.1128/AEM.72.2.1258-1266.2006.

Campbell, B.M., Vermeulen, S.J., Aggarwal, P.K., Corner-Dolloff, C., Girvetz, E., Loboguerrero, A.M., Ramirez-Villegas, J., Rosenstock, T., Sebastian, L., Thornton, P.K., and Wollenberg, E. 2016. Reducing risks to food security from climate change. Glob. Food Sec. **11**: 34–43. doi:10.1016/j.gfs.2016.06.002.

Carbonnel, S., and Gutjahr, C. 2014. Control of arbuscular mycorrhiza development by nutrient signals. Front. Plant Sci. **5**: 1–5. doi:10.3389/fpls.2014.00462.

Ceci, A., Pinzari, F., Russo, F., Maggi, O., and Persiani, A.M. 2018. Saprotrophic soil fungi to improve phosphate solubilisation and release: In vitro abilities of several species. Ambio 47.1: 30-40. DOI: 10.1007/s13280-017-0972-0.

Chou, L., Garrels, R.M., and Wollast, R. 1989. Comparative study of the kinetics and mechanisms of dissolution of carbonate minerals. Chem. Geol. **78**(3–4): 269–282. doi:10.1016/0009-2541(89)90063-6.

Christie, R.V. 2019. Going back to the soil: An integrated approach to farming. Wilfrid Laurier University. Available from https://scholars.wlu.ca/etd/2161.

Colin, Y., Nicolitch, O., Turpault, M.-P., and Uroz, S. 2017. Mineral types and tree species determine the functional and taxonomic structures of forest soil bacterial communities. Appl. Environ. Microbiol. **83**: e02684-16. doi:10.1128/AEM.02684-16.

Cordell, D., Drangert, J.-O., and White, S. 2009. The story of phosphorus: Global food security and food for thought. Glob. Environ. Chang. **19**(2): 292–305. doi:10.1016/j.gloenvcha.2008.10.009.

Demoling, F., Figueroa, D., and Bååth, E. 2007. Comparison of factors limiting bacterial growth in different soils. Soil Biol. Biochem. **39**(10): 2485–2495. doi:10.1016/j.soilbio.2007.05.002.

Diaz, R.J., and Rosenberg, R. 2008. Spreading dead zones and consequences for marine ecosystems. Science **321**: 926–929. doi:10.1126/science.1156401.

Doran, J.W., and Zeiss, M.R. 2000. Soil health and sustainability: managing the biotic component of soil quality. Appl. Soil Ecol. **15**(1): 3–11. doi:10.1016/S0929-1393(00)00067-6.

Ferris, F.G., and Lowson, E.A. 1997. Ultrastructure and geochemistry of endolithic microorganisms in limestone of the Niagara Escarpment. Can. J. Microbiol. **43**(3): 211–219. doi:10.1139/m97-029.

Food and Agriculture Organization of the United Nations. FAO Ecocrop profile for pea. Available from http://ecocrop.fao.org/ecocrop/srv/en/dataSheet?id=1721.

Food and Agriculture Organization of the United Nations. FAO Ecocrop profile for wheat. Available from http://ecocrop.fao.org/ecocrop/srv/en/dataSheet?id=2114.

Friese, C.F., and Allen, M.F. 1991. The spread of VA mycorrhizal fungal hyphae in the soil: inoculum types and external hyphal architecture. Mycologia **83**(4): 409. doi:10.2307/3760351.

Fuentes, J.P., Bezdicek, D.F., Flury, M., Albrecht, S., and Smith, J.L. 2006. Microbial activity affected by lime in a long-term no-till soil. Soil Tillage Res. **88**: 123–131. doi:10.1016/j.still.2005.05.001.

Gadd, G.M., and Griffiths, A.J. 1977. Microorganisms and heavy metal toxicity. Microb. Ecol. **4**(4): 303–317. doi:10.1007/BF02013274.

Goldich, S.S. 1938. A study in rock-weathering. J. Geol. **46**(1): 17–58. doi:10.1086/624619.

Gough, L.P., and Herring, J.R. 1993. Geologic research in support of sustainable agriculture. Agric. Ecosyst. Environ. **46**: 55–68. doi:https://doi.org/10.1016/0167-8809(93)90013-F.

Hacin, J.I., Bohlool, B.B., and Singleton, P.W. 1997. Partitioning of ^{14}C-labelled photosynthate to developing nodules and roots of soybean (Glycine max). New Phytol. **137**(2): 257–265. doi:10.1046/j.1469-8137.1997.00812.x.

Hameeda, B., Reddy, Y.H.K., Rupela, O.P., Kumar, G.N., and Reddy, G. 2006. Effect of carbon substrates on rock phosphate solubilization by bacteria from composts and macrofauna. Curr. Microbiol. **53**(4): 298–302. doi:10.1007/s00284-006-0004-y.

Harley, A.D., and Gilkes, R.J. 2000. Factors influencing the release of plant nutrient elements from silicate rock powders : a geochemical overview. Nutr. Cycl. Agroecosystems **56**: 11–36. doi:https://doi.org/10.1023/A:1009859309453.

Havlin, J., Beaton, J., Tisdale, S., and Nelson, W. 1999. Soil fertility and fertilizers, 6th edition. Prentice-Hall Inc, Upper Saddle River.

Heinrich, E.W.M. 1980. The geology of carbonatites. Rand McNally and Company, Huntington, US.

Hu, Z., Richter, H., Sparovek, G., and Schnug, E. 2004. Physiological and biochemical effects of rare earth elements on plants and their agricultural significance : a review. J. Plant Nutr. **27**: 183–220. doi:10.1081/PLN-120027555.

Hungate, B., Danin, A., Pellerin, N.B., Stemmler, J., Kjellander, P., Adams, J.B., and Staley, J.T. 1987. Characterization of manganese-oxidizing (MnII→MnIV) bacteria from Negev Desert rock varnish: implications in desert varnish formation. Can. J. Microbiol. **33**(10): 939–943. doi:10.1139/m87-165.

Jones, A.P., Genge, M., and Carmody, L. 2013. Carbonate melts and carbonatites. Rev. Mineral.

Geochemistry **75**: 289–322. doi:10.2138/rmg.2013.75.10.

Jones, J.M. 2016. Spanish River Carbonatite: Its benefits and potential use as a soil supplement in agriculture. Wilfrid Laurier University. Available from https://scholars.wlu.ca/etd/1795.

Jones, J.M., Webb, E.A., Lynch, M.D., Charles, T.C., Antunes, P.M., and Guinel, F.C. 2019. Does a carbonatite deposit influence its surrounding ecosystem? FACETS **4**: 389-406. doi:10.1139/facets-2018-0029.

Lauber, C.L., Hamady, M., Knight, R., and Fierer, N. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol. **75**(15): 5111–5120. doi:10.1128/AEM.00335-09.

Lehto, T. 1994. Effects of soil pH and calcium on mycorrhizas of *Picea abies*. Plant Soil **163**(1): 69–75. doi:10.1007/BF00033942.

Lodwig, E.M., Hosie, A.H.F., Bourdès, A., Findlay, K., Allaway, D., Karunakaran, R., Downie, J.A., and Poole, P.S. 2003. Amino-acid cycling drives nitrogen fixation in the legume–*Rhizobium* symbiosis. Nature **422**(6933): 722–726. doi:10.1038/nature01527.

Matson, P.A. 1997. Agricultural intensification and ecosystem properties. Science (80-.). **277**: 504–509. doi:10.1126/science.277.5325.504.

Myrvang, M.B., Heim, M., Krogstad, T., Almås, Å.R., and Gjengedal, E. 2017. The use of carbonatite rock powder as a liming agent. J. Plant Nutr. Soil Sci. **180**: 326–335. doi:10.1002/jpln.201600455.

Myrvang, M.B., Hillersøy, M.H., Heim, M., Bleken, M.A., and Gjengedal, E. 2016. Uptake of macro nutrients, barium, and strontium by vegetation from mineral soils on carbonatite and pyroxenite bedrock at the Lillebukt Alkaline Complex on Stjernøy, Northern Norway. J. Plant Nutr. Soil Sci. **179**(6): 705–716. doi:10.1002/jpln.201600328.

Philippot, L., Raaijmakers, J.M., Lemanceau, P., and van der Putten, W.H. 2013. Going back to the roots : the microbial ecology of the rhizosphere. Nat. Rev. Microbiol. Microbiol. **11**(11): 789–799. doi:10.1038/nrmicro3109.

Porter, W.M., Robson, A.D. and Abbott, L.K. 1987. Factors controlling the distribution of vesicular-arbuscular mycorrhizal fungi in relation to soil pH. J. Appl. Ecol. **24**: 663-672. DOI: 10.2307/2403901.

Raynaud, X., and Nunan, N. 2014. Spatial ecology of bacteria at the microscale in soil. PLoS One **9**(1): e87217. doi:10.1371/journal.pone.0087217.

Reddy, M.S., Kumar, S., Babita, K., and Reddy, M.S. 2002. Biosolubilization of poorly soluble rock phosphates by *Aspergillus tubingensis* and *Aspergillus niger*. Bioresour. Technol. **84**(2): 187–189. doi:10.1016/S0960-8524(02)00040-8.

Rogers, J.R., and Bennett, P.C. 2004. Mineral stimulation of subsurface microorganisms: release of limiting nutrients from silicates. Chem. Geol. **203**(1–2): 91–108. doi:10.1016/j.chemgeo.2003.09.001.

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., and Fierer, N. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. Int. Soc. Microb. Ecol. J. **4**: 1340–1351. doi:10.1038/ismej.2010.58.

Sage, R.P. 1987. Spanish River Carbonatite Complex. Ontario Ministry of Northern Development and Mines, Toronto, CA.

Savard, M.M., Somers, G., Smirnoff, A., Paradis, D., van Bochove, E., and Liao, S. 2010. Nitrate isotopes unveil distinct seasonal N-sources and the critical role of crop residues in groundwater contamination. J. Hydrol. **381**(1–2): 134–141. doi:10.1016/j.jhydrol.2009.11.033.

Shehata, H.R., Dumigan, C., Watts, S., and Raizada, M.N. 2017. An endophytic microbe from an unusual volcanic swamp corn seeks and inhabits root hair cells to extract rock phosphate. Sci. Rep. **7**(1): 13479. Springer US. doi:10.1038/s41598-017-14080-x.

Suwa, R., Jayachandran, K., Nguyen, T.N., Abdellah, B., Fujita, K., and Saneoka, H. 2008. Barium toxicity effects in soybean plants. Arch. Environ. Contam. Toxicol. **55**: 397–403. doi:10.1007/s00244-008-9132-7.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., and Polasky, S. 2002. Agricultural sustainability and intensive production practices. Nature **418**: 671–677. doi:10.1038/nature01014.

Timmers, A.C.J., Soupène, E., Auriac, M.-C., de Billy, F., Vasse, J., Boistard, P., and Truchet, G. 2000. Saprophytic intracellular rhizobia in alfalfa nodules. Mol. Plant-Microbe Interact. **13**(11): 1204–1213. doi:10.1094/MPMI.2000.13.11.1204.

Tyler, G. 2004. Rare earth elements in soil and plant systems - A review. Plant Soil **267**: 191–206. doi:10.1007/s11104-005-4888-2.

Uroz, S., Calvaruso, C., Turpault, M., and Frey-Klett, P. 2009. Mineral weathering by bacteria: ecology, actors and mechanisms. Trends Microbiol. **17**(8): 378–387. doi:10.1016/j.tim.2009.05.004.

Uroz, S., Calvaruso, C., Turpault, M.P., Pierrat, J.C., Mustin, C., and Frey-Klett, P. 2007. Effect of the mycorrhizosphere on the genotypic and metabolic diversity of the bacterial communities involved in mineral weathering in a forest soil. Appl. Environ. Microbiol. **73**(9): 3019–3027. doi:10.1128/AEM.00121-07.

Uroz, S., Kelly, L.C., Turpault, M., Lepleux, C., and Frey-Klett, P. 2015. The mineralosphere concept : mineralogical control of the distribution and function of mineral-associated bacterial communities. Trends Microbiol. **23**(12): 751–762. doi:10.1016/j.tim.2015.10.004.

van Schöll, L., Kuyper, T.W., Smits, M.M., Landeweert, R., Hoffland, E., and van Breemen, N. 2008. Rock-eating mycorrhizas : their role in plant nutrition and biogeochemical cycles. Plant Soil **303**: 35–47. doi:10.1007/s11104-007-9513-0.

van Straaten, P. 2002. Rocks for Crops: agrominerals of sub-Saharan Africa. ICRAF, Nairobi, Kenya.

van Straaten, P. 2006. Farming with rocks and minerals: challenges and opportunities. An. Acad. Bras. Cienc. **78**(4): 731–747. doi:10.1590/S0001-37652006000400009.

van Straaten, P. 2007. Agrogeology: The use of rocks for crops. Enviroquest Ltd., Toronto, CA.

VanVolkenburg, H. 2019. Exploring diversified vineyard ecological soil management strategies: Impacts of cover cropping, Spanish River Carbonatite, and smooth pigweed (*Amaranthus hybridus*) interactions on an agroecosystem. Brock University. Available from; https://dr.library.brocku.ca/handle/10464/14449.

Vaughan, R.G., Kervyn, M., Realmuto, V., Abrams, M., and Hook, S.J. 2008. Satellite measurements of recent volcanic activity at Oldoinyo Lengai, Tanzania. J. Volcanol. Geotherm. Res. **173**: 196–206. doi:10.1016/j.jvolgeores.2008.01.028.

Whitman, T., Neurath, R., Perera, A., Chu-Jacoby, I., Ning, D., Zhou, J., Nico, P., Pett-ridge, J., and Firestone, M. 2018. Microbial community assembly differs across minerals in a rhizosphere

microcosm. Environ. Microbiol. **20**: 4444–4460. doi:10.1111/1462-2920.14366.

Woolley, A., and Kempe, D. 1989. Carbonatites: nomenclature, average chemical compositions, and element distribution. *In* Carbonatites: Genesis and evolution. *Edited by* Bell K. Unwin Hyman, London. pp. 1–14.

Woolley, A., and Kjarsgaard, B. 2008a. Paragenetic types of carbonatite as indicated by the diversity and relative abundances of associated silicate rocks: evidence from a global database. Can. Mineral. **46**: 741–752. doi:10.3749/canmin.46.4.741.

Woolley, A.R., and Kjarsgaard, B.A. 2008b. Carbonatite occurrences of the world: map and database. Geological Survey of Canada Open File # 5796.

Xu, C., Wang, L., Song, W., and Wu, M. 2010. Carbonatites in China : A review for genesis and mineralization. Geosci. Front. **1**: 105–114. doi:10.1016/j.gsf.2010.09.001.

Zapata, F., and Roy, R. 2004. Use of phosphate rocks for sustainable agriculture. *In* FAO Fertilizer and Plant Nutrition Bulletin. Food and Agriculture Organization of the United Nations (FAO).

Zhang, G., Kang, J., Wang, T., and Zhu, C. 2018. Review and outlook for agromineral research in agriculture and climate mitigation. Soil Res. **56**(2): 113. doi:10.1071/SR17157.

Zhong, W., Gu, T., Wang, W., Zhang, B., Lin, X., Huang, Q., and Shen, W. 2010. The effects of mineral fertilizer and organic manure on soil microbial community and diversity. Plant Soil **326**(1–2): 511–522. doi:10.1007/s11104-009-9988-y.

Chapter 3 - Does a carbonatite deposit influence its surrounding ecosystem?

In this publication, the soils, plants, and soil microorganisms overlying a carbonatite deposit in northern Ontario, Canada, were assessed to determine what impacts the carbonatite was having on the ecosystem. It has been reproduced from the final proofs submitted to FACETS during publication. The authors retain their copyright over the published material following FACETS copyright policy (https://www.facetsjournal.com/about/faq/). It was published August 2019 with the DOI: 10.1139/facets-2018-0029.

Author contributions:

JMCJ, PMA, and FCG conceived and designed the study. JMCJ, MDJL, and TCC performed the experiments/collected the data. JMCJ, EAW, MDJL, PMA, and FCG analyzed and interpreted the data. PMA, FCG, and TCC contributed resources. JMCJ, EAW, PMA, and FCG drafted or revised the manuscript.

Does a carbonatite deposit influence its surrounding ecosystem?

James MC Jones*[1], Elizabeth A Webb[2], Michael DJ Lynch[3,4], Trevor C Charles[3,4], Pedro M. Antunes[5], Frédérique C Guinel[1].

1 – Department of Biology, Wilfrid Laurier University, Waterloo, Ontario, Canada

2 – Department of Earth Sciences, University of Western Ontario, London, Ontario, Canada.

3 – Department of Biology and Waterloo Centre for Microbial Research, University of Waterloo, Waterloo, Ontario, Canada

4 – Metagenom Bio Inc., Toronto, Ontario, Canada

5 – Department of Biology, Algoma University, Sault Ste. Marie, Ontario, Canada

* Corresponding author, contact information: jone3630@mylaurier.ca

Abstract

Carbonatites are unusual alkaline rocks with diverse compositions. Although previous work has characterized the effects these rocks have on soils and plants, little is known about their impacts on local ecosystems. Using a deposit within the Great Lakes–St. Lawrence forest in northern Ontario, Canada, we investigated the effect of a carbonatite on soil chemistry and on the structure of plant and soil microbial communities. This was done using a vegetation survey conducted above and around the deposit, with corresponding soil samples collected for determining soil nutrient composition and for assessing microbial community structure using 16S/ITS Illumina Mi-Seq sequencing. In some soils above the deposit a soil chemical signature of the carbonatite was found, with the most important effect being an increase in soil pH compared with the non-deposit soils. Both plants and microorganisms responded to the altered soil chemistry: the plant communities present in carbonatite-impacted soils were dominated by ruderal species, and although differences in microbial communities across the surveyed areas were not obvious, the abundances of specific bacteria and fungi were reduced in response to the carbonatite. Overall, the deposit seems to have created microenvironments of relatively basic soil in an otherwise acidic forest soil. This study demonstrates for the first time how carbonatites can alter ecosystems in situ.

Introduction

Carbonatites are a diverse and unusual group of igneous rocks defined by Woolley and Kempe (1989) as containing ≥50% carbonate minerals by volume. There are at least 527 identified carbonatite deposits worldwide, and information on the mineralogy of 477 of these sites is available (Woolley and Kjarsgaard 2008a). The majority (93%) of described deposits have been dated to 2.5–4.0 billion years old (Jones et al. 2013) and these are found in Africa (36%), Asia (34%), and North America/Greenland (23%). Economically, carbonatites are usually considered exclusively as a source of rare earth elements (REE) and trace elements, though several deposits of economic value also contain agriculturally-relevant minerals such as calcite ($CaCO_3$) or dolomite ($CaMg(CO_3)_2$) (Woolley and Kjarsgaard 2008b; Myrvang et al. 2016). Carbonatite rocks frequently contain various amounts of the Ca-phosphate mineral apatite and the K-bearing phyllosilicates biotite and phlogopite and so carbonatites have been considered as an alternative source of these nutrients for plants (Heinrich 1980). Additionally, carbonatites are a source of high-grade phosphate minerals, especially in Brazil and South Africa, and are thus important sources of this element for agriculture (Mariano 1989). More recently, carbonatite rock mined from a deposit in northern Ontario, Canada, has been the focus of preliminary studies exploring its effectiveness as a natural mineral fertilizer (Jones 2016). However, further work is needed to understand what the costs and benefits might be of using minimally processed carbonatite rocks and minerals in agricultural settings.

The impact of these rocks on soil geochemistry has been the focus of several previous studies. Because carbonatite deposits are rich in REE and phosphorus, prospectors have used geochemical fingerprinting in soils and stream sediments to locate buried carbonatite deposits (e.g., Kunzendorf and Secher 1987; Ahn et al. 2014). Vestin et al. (2006) explored the Alnö carbonatite deposit in Sweden with the aim of determining the influence of the alkaline deposit on the chemistry of the overlying soils (0–30 cm depths) and found that there was a distinct geochemical signature characterizing the deposit's influence throughout the surveyed soil horizons. This was followed up by a later study (Vestin et al. 2013) where it was concluded that the Norway spruce (*Picea abies* (L.) H.Karst.)) growing in the alkaline soils above the deposit had a higher growth rate and increased tissue Ca content than those trees in less alkaline soils not affected by the deposit. A similar study was done by Myrvang et al. (2016) who investigated nutrient content of the vegetation overlying the Stjernøy carbonatite deposit in Norway. Although the plant content of several elements (Ca, Mg, P, and K) indicated that the vegetation above the deposit was obtaining adequate if not higher levels of nutrients, the ecological significance of this finding was not explored. Hanslinger et al. (2007) reported that the Alnö carbonatite deposit in Sweden is better delineated by the unique vegetation assemblages supported by the alkaline soils above the deposit than by the existing geological map. This relates to the primary aim of geobotanical prospecting, whereby underlying geological formations are identified based on the overlying vegetation (Brooks 1972). Though difficulties in this

technique arise from the expertise required (e.g., to identify the plants) and the sometimes-broad habitat preferences of potential indicator species (Moon 2006), it can be used with some success in situations where strong geological boundaries exist. For instance, differences in the types and growth of vegetation above silicate bedrock and carbonatite bedrock were identified in a Finnish study (Talvitie 1979). Although these diverse studies provide evidence that plant growth is improved or altered with carbonatite presence, it is largely unclear how vegetation communities and soil microorganisms may respond to the presence of these rocks. Weathering of minerals from carbonatite deposits increases nutrient content and pH of the overlying soil and it is expected that because of these abiotic changes carbonatite deposits will also influence the biota within the soil and ultimately the larger ecosystems based around those soils. Indeed, in a recent study by Colin et al. (2017), it was demonstrated that when calcite and apatite were introduced to tree stands there were compositional shifts in the soil bulk bacterial community. These minerals are abundant in many carbonatites (Woolley and Kjarsgaard 2008a). Yet, to our knowledge, no study has investigated how the presence of carbonatite rocks affects the overlying soil microbiota.

Here, we examined the Spanish River Carbonatite (SRC) deposit near Sudbury, Ontario, Canada in its capacity to affect the surrounding ecosystem. More specifically, we investigated whether the SRC causes distinct changes in the biotic and abiotic factors of the ecosystem overlying the deposit by establishing comparisons within and outside the deposit. First, we identified whether the glacial till soils above the deposit were distinct from those present in the area outside the deposit (i.e., whether the soils overlying the deposit had a chemical signature indicative of SRC influence). Second, we assessed whether plant community composition could be explained by differences in soil chemistry in and around the deposit. Finally, we determined the composition of the soil microbial communities, and whether changes based on SRC presence/absence were obvious.

Materials and methods

Geological history and deposit description

The SRC deposit is located south of the Spanish River Provincial Park (UTM: 17T 0444556 5163894). The area is part of the Great Lakes–St. Lawrence forest region, and contains a mixed forest primarily composed of jack pine (*Pinus banksiana* Lamb.), red pine (*Pinus resinosa* Aiton), and trembling aspen (*Populus tremuloides* Michx.). This carbonatite deposit was formed by intrusive igneous activity into quartz monzonite bedrock 1.84 billion years ago (Sage 1987). Because of the similarity in age between the carbonatite deposit and the nearby Sudbury Impact Crater (also dated to 1.84 billion years ago; Petrus et al. 2015), the deposit may have arisen due to geological instability from the impact. The most recent glaciation period in the area was ~13 000 years ago (Dyke et al.

2002). The glacial activity left till of varying depths across the area that somewhat isolates the SRC deposit from the surface. The overburden depth ranges from 0 m at the quarry to at least ~56 m near the outer core according to borehole exploration by Agricultural Mineral Prospectors Inc. (2004). The SRC deposit has an approximate surface area of 3.25 km^2, with distinct mineralogical zones (Figure 3.1; Sage 1987) referred to here as the inner core (IC), outer core (OC), and transition zone (TZ). Based on a geological survey done by Agricultural Mineral Prospectors Inc. (2000), the IC carbonatite is composed primarily of calcitic carbonatite with apatite, abundant to minor amounts of vermiculite, and minor amounts of iron oxide and magnetite. The OC carbonatite consists of calcitic carbonatite interbanded with fenite with abundant to moderate amounts of vermiculite, and some iron oxide and apatite. The TZ is predominantly fenite consisting of altered granodiorite with minor amounts of iron oxide and calcitic carbonatite. For this study, two additional zones were designated and used as positive and negative controls, respectively: the quarry (Q) zone within the OC where the SRC deposit is exposed, and the area outside the deposit (OU). Each zone was sampled twice in different areas for a total of 10 sites (Figure 3.1).

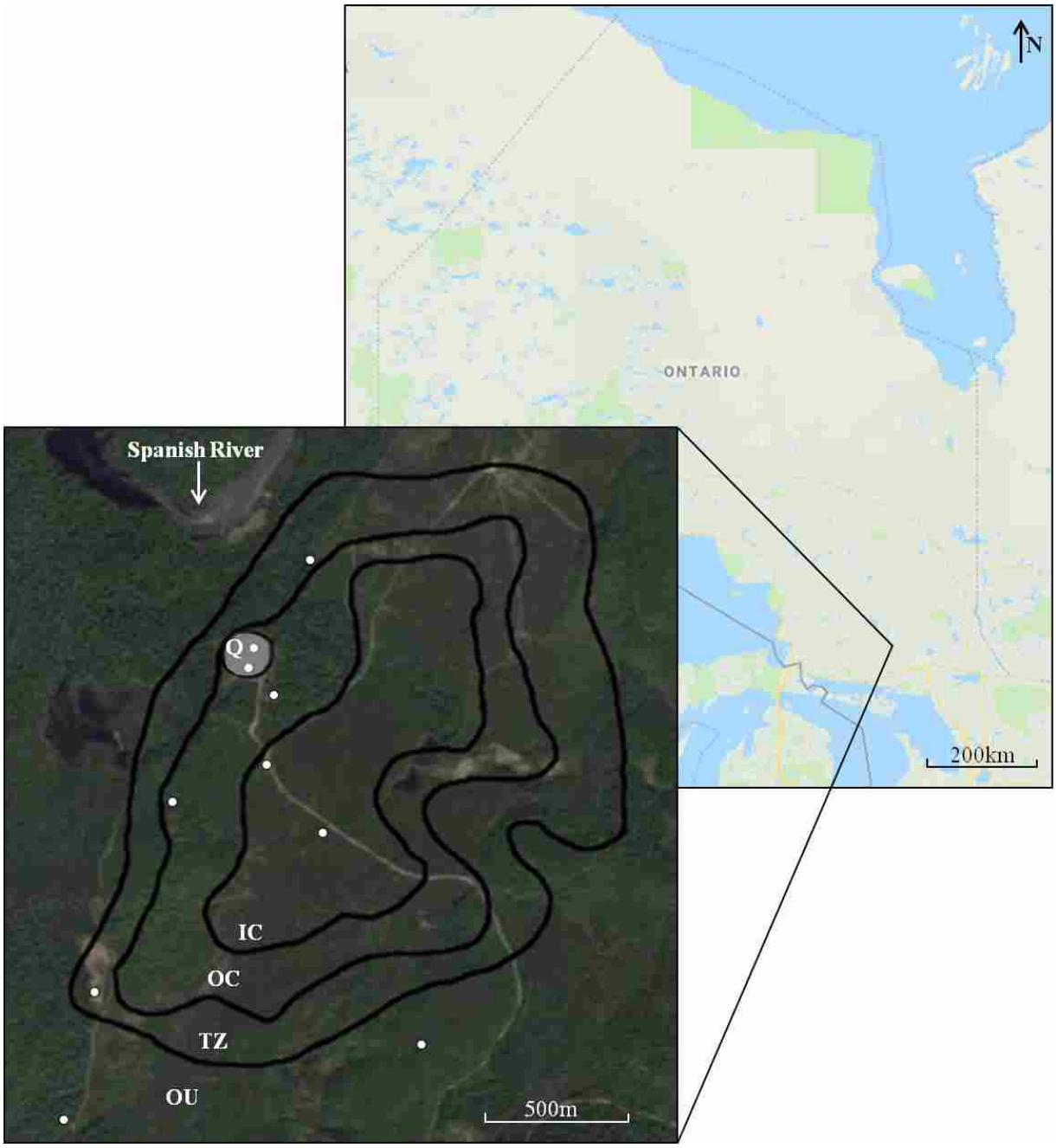


Figure 3.1: Overview of the Spanish River Carbonatite (SRC) deposit located near the Spanish River in Ontario, Canada. Five zones were considered for the study and are delineated by the black lines: the quarry (grey circle) where SRC is actively mined (Q), the inner core (IC), the outer core (OC), the transition zone (TZ), and the outside of the deposit (OU). Sampling sites are indicated by the white dots. Zones estimated from data provided by Agricultural Mineral Prospectors Inc. (2000). Map data provided by Google (2018) and satellite data provided by DigitalGlobe and CNES/Airbus (2018).

Vegetation and soil sampling

A modified-Whittaker plot (Stohlgren et al. 1995) was used to assess the species abundance and percent cover of vegetation at each site, including trees. Specific logging/reforestation data were unavailable for the area, and so disturbance was estimated for each site. With the exception of one site dominated by jack pine in the IC that was likely reforested some time ago, the areas surveyed for this project appeared to be either undisturbed or old growth forest. Prior to including the disturbed site in the dataset, it was compared with the other IC site to determine whether the reforestation had altered the vegetation in the area. Sampling was undertaken in mid-August of 2015. Species were identified in the field according to descriptions by Dickinson et al. (2004) and Newcomb (1989). Unknown species were collected and later identified using the Northern Ontario Plant Database (northernontarioflora.ca/), with pressed samples deposited at the Algoma University herbarium.

Whole root systems were collected from individuals of the three most abundant herbaceous plant species in each plot for assessment of colonization by arbuscular–mycorrhizal fungi. Roots of plants known to be mycorrhizal (following the list by Wang and Qiu 2006) were stained using Trypan blue (Brundrett et al. 1996), and colonization quantified using the gridline intersect method of McGonigle et al. (1990). Soil samples were collected from the centre of each of the modified-Whittaker 10 m^2 subplots (two subplots per site). An open-faced auger was used to collect soil until reaching the C horizon or a maximum depth of 80 cm. Aside from the exposed material at the quarry, bedrock material was not encountered. Soil cores were placed sequentially on a 70% ethanol-sterilized portable whiteboard and each soil horizon was identified by differences in colour and texture. Where soil horizons could not be visually identified in situ, they were classified according to their placement in the soil profile (e.g., the first non-organic horizon was considered to be horizon A). At the quarry, both exposed bedrock and a residual soil (at least 40 cm deep) were present. The soil had formed directly on the weathered bedrock and consisted of small rock fragments generally <3 mm in size that responded strongly to HCl. This soil was supporting sporadic small plants at the time of sampling, and desiccation cracks were present in low-lying sections. In the soil profile of the sampled areas in the quarry, the uppermost horizon consisted of SRC (distinguished as weathered by lighter visual appearance) and small amounts (≤0.6%) of organic matter, whereas the lower horizon consisted of unweathered (or less-weathered/darker-coloured) SRC and trace (0.2%) organic matter. For comparisons in modelling, these were labelled as the A and B horizons, respectively. At each site and when possible, two samples were collected from each horizon in sterile 50 mL Falcon™ tubes: one was taken using axenic techniques and stored at 4 °C for analysis of soil microbial community composition (determined by Metagenom Bio Inc., Toronto, Ontario, Canada), and the other was collected for soil chemical analyses (conducted by Actlabs Agriculture Division, Ancaster, Ontario, Canada). The soil chemical parameters determined were pH, organic matter (OM) content, nutrient content (P, K, Ca, Mg, Na, S, Zn, Mn, B, Cu, Fe, Al, and total N), and cation exchange capacity

(CEC). The concentrations of the elements were determined by inductively coupled plasma optical emission spectroscopy following Mehlich-3 extraction. The CEC was calculated from the elemental concentration values, and the total N/OM determined by combustion analysis. Finally, the texture, percent coarse fragments, fizzing response to 10% HCl, and colour of the soils were noted in situ. A total of 78 soil samples were collected for microbial community composition and a total of 75 for soil chemical analyses.

Microbial community DNA isolation and 16S/ITS sequencing

DNA was isolated from each soil sample using a Norgen Soil DNA Isolation Kit following the supplier's recommendation. For bacteria, the V3–V4 region of the 16S rRNA was selectively amplified using the 340F/806R primers (Takahashi et al. 2014), whereas for fungi the Internal Transcribed Spacer 1 (ITS1) region was amplified using the BITS1/B58S3 primers (Bokulich and Mills 2013). The PCR cycle used for amplification was as follows, with each sample run in triplicate and pooled following amplification: initial denaturing at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 45 °C for 30 s, 68 °C for 50 s and final extension at 68 °C for 10 min. Amplicon size and amount were confirmed by visualizing 2 µL on a 2% TAE agarose gel. Once the amount of 16S/ITS1 rRNA was confirmed, pooled rRNA was gel purified and quantified using Qubit dsDNA HS Assay Kit following the manufacturer's instructions. Library DNA was prepared according to the MiSeq System Denature and Dilute Libraries Guide (Illumina Document #15039740 v01). A 6 pM library with 5% PhiX was sequenced with MiSeq Reagent Kit (v2), and the MiSeq data were streamed to BaseSpace. Although 78 samples were processed, only 71 samples for 16S and 70 samples for ITS were of sufficient quality for successful sequence generation. The mean depth was 25 689 reads for 16S samples and 28 808 reads for ITS samples. Sequences (with primers removed) were assembled with pandaseq (version 2.11; Masella et al. 2012) with a quality threshold of 0.7. Chimera detection/removal and clustering of sequences at 97% identity were accomplished with UPARSE (version 9; Edgar 2013) with singleton sequences removed. The 16S representative sequences were classified using the RDP Classifier (Wang et al. 2007) trained against the Greengenes database (version 13_8) with a 0.8 classification threshold (RDP Classifier bootstrap), whereas the ITS representative sequences were classified against the UNITEdb (version 7.1) using UCLUST (Edgar 2010). The operational taxonomic unit (OTU) representative sequence set was aligned using either the PyNAST algorithm (Caporaso et al. 2010) against the Greengenes (16S) or MUSCLE (version 3.8.31; Edgar 2004; (ITS)) and a representative phylogeny was constructed using FastTree 2.1.3 SSE3 (Price et al. 2010) with the GTR model. Sequence data were deposited in the NCBI database (submission number SUB3462412).

Statistical analysis and bioinformatics

Our first question was whether the chemistry of soils within the deposit was distinct from that of the soils outside of the deposit, and possessed a chemical signature indicative of SRC influence. Because a large number of soil chemical variables had near-linear or linear distributions, a principal component analysis (PCA) was used to identify differences in chemistries across the sampled areas. For the PCA, the soil horizons were not considered (individual horizon data are available in Table S3.1). This test was followed by analyses of variance (ANOVAs) to validate whether soil chemical factors could be used to group samples according to presumed SRC influence. Post hoc Tukey's HSD tests were conducted to confirm differences in soil chemical properties across groups. Our next question was whether differences in plant community composition could be explained by observed differences in soil chemistry. To answer this question, constrained ordination with redundancy analysis (RDA) was undertaken with the presence/absence data of plant species from the 1 m^2 modified-Whittaker subplots (excluding mature trees). Data from the Q sites were not included in this analysis because the area is actively mined and therefore highly disturbed. Because soil chemical analyses were not conducted for the 1 m^2 subplots, the mean values from the 10 m^2 subplots were used. Analysis was done using the R "vegan" package (version 2.4-1; Oksanen et al. 2016), specifically the "rda" function for RDA and the "anova.cca" function (1500 permutations) for the random permutation test. Additionally, the Shannon diversity indices were calculated (Fowler et al. 1998) for each sub-plot in the modified-Whittaker plot.

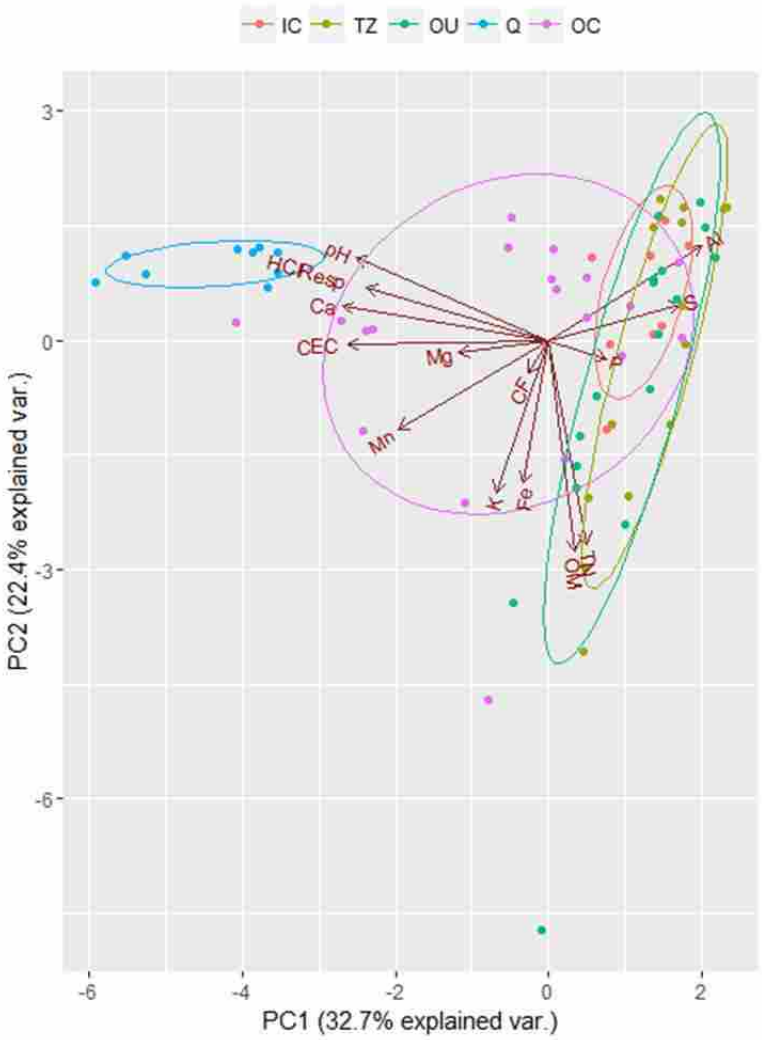
Our last question was whether differences in soil chemistry affected bacterial (16S) and fungal (ITS) communities, which was addressed using two methods: non-metric multi-dimensional scaling (NMDS) coupled with a permutational multivariate analysis of variance (PERMANOVA) to quantify changes in community composition, and differential abundance analysis to identify indicator OTUs. Bacterial and fungal community composition was determined with core samples pooled (i.e., without considering horizons). Prior to the analysis, OTUs with low read numbers (<5) were removed and abundances converted to relative proportions. Differential abundance was evaluated using the DeSeq2 R package (version 1.12.4; Love et al. 2014), with the geometric means correction for data containing zeros. The PERMANOVA analysis was undertaken using the vegan R package and the "adonis" function with a Bray–Curtis distance matrix. When possible, the differentially abundant OTUs were further classified by an NCBI nucleotide BLAST search for highly similar sequences against the NCBI nucleotide collection (≥99% similarity) excluding uncultured and environmental sequences. All data analyses were conducted in the R software environment (version 3.3.1; R Core Team 2016).

Results

Soil chemistry differed with overburden depth and only weakly across zones

It was expected that the different mineralogy of bedrock in the zones within the SRC deposit would give rise to unique soil chemical parameters in each of these areas. Although the sampling sites partially grouped by zone in the PCA (Figure 3.2), some areas within the deposit (e.g., IC) had nearly indistinguishable soil chemistries from those areas outside the deposit. Overall, soils from the IC, TZ, and OU zones grouped together, the Q soils were the most different, and the OC soils grouped between those two sets.

According to the ordination vectors and the loadings for the main axis of sample separation (Figure 3.2; PC1), the primary differences among soil samples, in order of contribution to PC1 and irrespective of sign, were seen in CEC, Ca, Al, Mg, S, Mn, pH, and P. Using the differences in these parameters, a SRC influence signature was established and used to reclassify the sampling areas into three new groupings: areas of high SRC influence (Q), areas of moderate SRC influence (OC), and areas of low/negligible SRC influence (IC, TZ, OU). These groups were validated by comparing the soil found outside of the deposit (low/negligible influence) to the "pure" SRC at the quarry (high influence) using a one-way ANOVA as above (Figure 3.3). Areas of high SRC influence were characterized by increased levels of Ca, Mn, and higher CEC and pH (Figs. 3a–3d, respectively) when compared to low/negligible influence sites. The moderate influence sites were most similar to the high influence sites with regards to those elements, but usually did not reach the same levels. When assessed across horizons, soil pH was typically within 0.2 units of any other horizon within influence categories (Table 3.1). There was, however, a large difference in soil pH between the high, moderate, and low/negligible SRC sites, with them being basic (~pH 7.60), moderately acidic (~pH 5.85), and acidic (~pH 4.85), respectively (Table 3.1). The areas of low SRC influence were characterized by increased Al and S content compared to those placed in the moderate and high categories (Figs. 3e and 3f, respectively). Finally, the soil Mg (Figure 3.3g) and P (Figure 3.3h) contents were not indicative of influence category. The soils across sites could not be differentiated based on their physical characteristics; they had a similar texture, HCl response, and colour, with the exception of the soil at the Q sites which were almost entirely formed of carbonatite. Following the above results, the SRC influence categories were used for the later analyses where applicable (e.g., PERMANOVA). The soil chemistry of the IC site that was thought to have been disturbed by reforestation was not different from that of the other IC site sampled.



Factor	PC1	PC2
CF	0.05405	-0.18224
HClResp	-0.10944	0.14089
pH	-0.19101	0.37768
OM	-0.20465	-0.46285
P	-0.05783	-0.05528
K	-0.26669	-0.23410
Ca	-0.38710	0.29063
Mg	-0.36934	0.27767
S	0.27871	-0.05856
Mn	-0.25092	-0.26221
Fe	-0.23393	-0.23748
Al	0.37919	0.07421
CEC	-0.42341	0.12457
TN	-0.16608	-0.47014

Figure 3.2: Principal component analysis of soil chemistry factors coloured by zone. The table to the right contains the loadings of each soil factor to the first two components. IC, inner core; TZ, transition zone; OU, outside of the deposit; Q, quarry; OC, outer core; CF, coarse fragments (%); OM, organic matter content (%); CEC, cation-exchange capacity (meq/100 g); TN, total nitrogen (%).

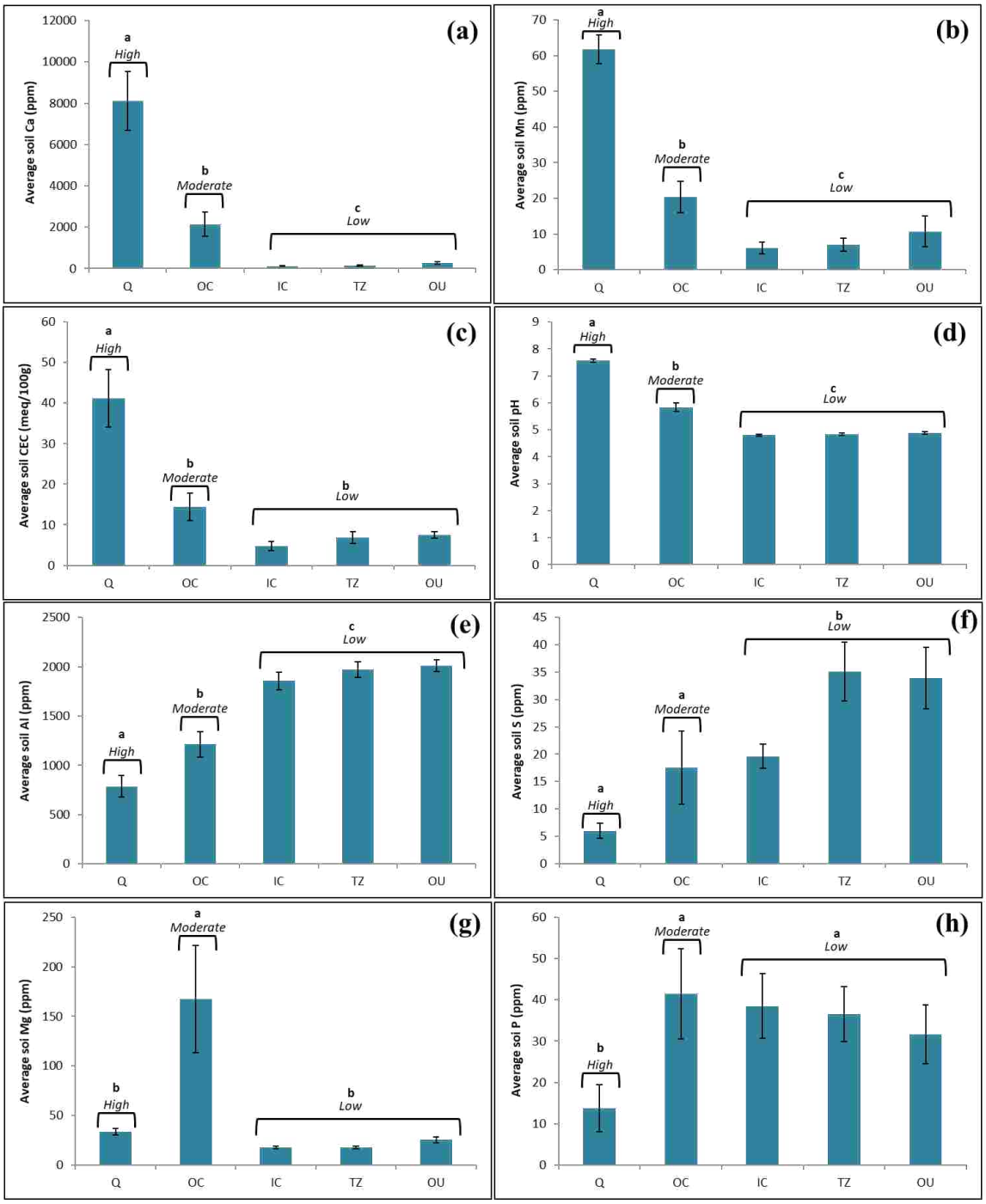


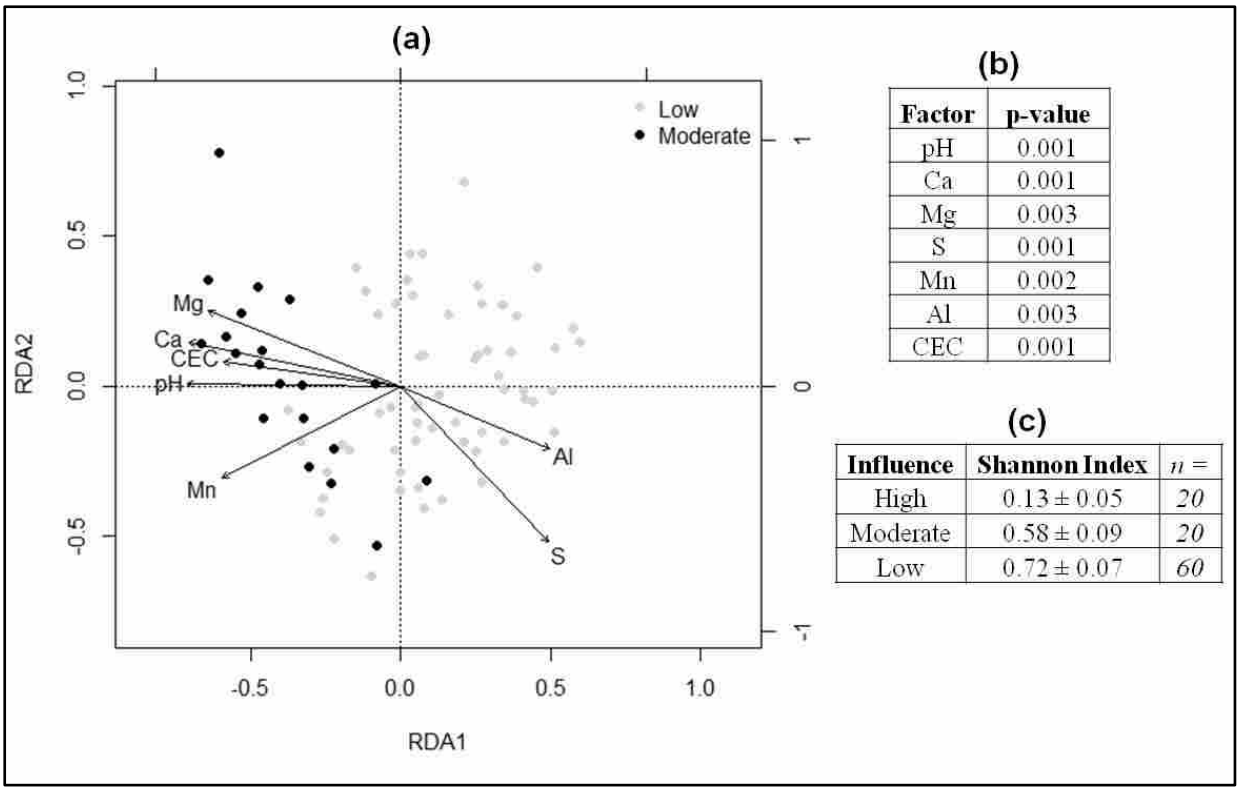
Figure 3.3: Mean soil chemistry values across zones and high, moderate, and low/negligible Spanish River Carbonatite (SRC)-influence categories. Deposit zones were assigned to influence categories based on similarities in their soil chemistry values (confirmed by statistical analysis) and are indicated by the above brackets. The Ca, Mn, S, Mg, and P values were log-transformed before statistical analysis. Chemistry values that differ significantly between SRC-influence categories (one-way ANOVA, 95% confidence level) are indicated with different lower-case letters. CEC, cation-exchange capacity.

Table 3.1: Mean (± standard error) soil pH for each of the major soil horizons (A, B, C) separated by low/negligible, moderate, and high SRC-influence. n = 2-11 samples per SRC-influence category per horizon.

Soil Horizon	Low	Moderate	High
A	4.80 ± 0.06	5.80 ± 0.27	7.60 ± 0.07
B	4.85 ± 0.04	5.85 ± 0.12	7.63 ± 0.12
C	4.89 ± 0.04	5.88 ± 0.33	7.40 ± 0.10

Plant community composition varied with soil chemical properties

Separation of plant communities in the RDA was driven by increases in pH, Ca, Mg, CEC, and Mn (Figure 3.4a). Constraining the ordination by soil chemical factors explained 26.0% of the variation in community composition, and all the tested soil factors were shown to equally contribute to community variation as shown by the permutation test output (Figure 3.4b). In sites of moderate SRC influence (i.e., within the OC), the abundant plant species (i.e., defined as ≥3 individuals across the two tested sites within a zone) consisted of *Acer spicatum* Lamarck, *Solidago altissima* Linnaeus, *Thuja occidentalis* Linnaeus, *Acer rubrum* Linnaeus, *Rubus idaeus* ssp. *strigosus* (Michaux) Focke, and *Comptonia peregrina* (Linnaeus) Coulter. In sites of low/negligible SRC influence (i.e., the IC, TZ, and OU), the plant species that were abundant (i.e., found in at least two of the zones) and unique to these areas consisted of *Maianthemum canadense* Desfontaines, *Vaccinium angustifolium* Bentham, *Pleurozium schreberi* (Bridel-Brideri) Mitten, *Pteridium aquilinum* (Linnaeus) Kuhn, and *Lysimachia borealis* (Rafinesque-Schmaltz) Manns & Anderberg. Although several abundant species were present in both the moderate and low/negligible SRC influence sites, the only abundant species present across all areas was *Cornus canadensis* Linnaeus, which had approximately 10 individuals in each of the IC, TZ, and OU, but only three individuals in the OC sites. The Shannon diversity indices for the 1 m^2 subplots suggested an increase in plant diversity/abundance as SRC influence decreased (Figure 3.4c). As the most abundant species in each zone were typically different, meaningful comparisons of mycorrhizal colonization could not be conducted (Table S3.2). Analysis of the growth of mature trees also could not be completed because the dominant tree species in each area were typically different (Table S3.3); it was further complicated by reforestation which may have occurred near the deposit. The vegetation community of the disturbed site did not appear different from that of the other IC site during ordination.



(b)

Factor	p-value
pH	0.001
Ca	0.001
Mg	0.003
S	0.001
Mn	0.002
Al	0.003
CEC	0.001

(c)

Influence	Shannon Index	*n* =
High	0.13 ± 0.05	*20*
Moderate	0.58 ± 0.09	*20*
Low	0.72 ± 0.07	*60*

Figure 3.4: (a) RDA biplot with samples shaded by Spanish River Carbonatite (SRC)-influence categories. Species presence/absence from the 1 m2 modified-Whittaker subplots were used as the vegetation data, and the averaged soil chemistry variables from the 10 m2 subplots used as the environmental chemistry data. The percent variation explained by the soil factor constraints is 26.0%. (b) Results of the soil chemistry permutation test to determine significant contributions to plant community structure. For the permutation test, 1500 permutations were used with "set.seed(2)". (c) Average Shannon diversity indices from the 1 m2 subplots with the standard error and number of samples for each SRC influence category. CEC, cation-exchange capacity (meq/100 g).

SRC impacted specific microorganisms but not overall communities

When all horizons were pooled, the bacterial communities showed weak grouping by SRC influence category (Figure 3.5a). The PERMANOVA indicated a significant influence of SRC (p-value = 0.021) on bacterial community composition, although the variation explained by this model was very low (R^2 = 0.0766). Between the high and moderate SRC influence sites, no bacterial OTUs were found to be significantly different in abundance, and between the moderate and low categories, only one OTU was significantly more abundant in the sites of moderate influence. This OTU was identified to the Micrococcaceae family (Phylum Actinobacteria). The BLAST search was able to further narrow down the identity of this OTU, with 100% identity match to several species within both the *Arthrobacter* and *Pseudoarthrobacter* genera (*e*-value = 0.0). Between the low and high SRC influence sites, there were a total of 28 bacterial OTUs that were of differential abundance (Table 2.2). Only two of these OTUs, the aforementioned Micrococcaceae and a Gaiellaceae family member (Phylum Actinobacteria), were more abundant in sites of high SRC influence. The rest were of higher abundance in the low/negligible SRC influence sites. For the Gaiellaceae, the BLAST search narrowed the identity down to *Gaiella occulta* strain F2-233 (95% identity, *e* -value = 0.0, accession: NR118138.1).

For the fungi, it was unclear whether there was any grouping based on SRC influence level (Figure 3.5b). Though the PERMANOVA indicated that SRC was a significant driver of community differences (p = 0.032), the variation explained by the model was quite low (R^2 = 0.0498). Several fungal OTUs were found to be differentially abundant between the SRC influence categories: a *Tylospora* species (Phylum Basidiomycota), a *Thelephoraceae* species (Phylum Basidiomycota), a *Leotiomycetes* member (Phylum Ascomycota), and two OTUs that could not be assigned to a class rank. A BLAST search was able to identify one of the unassigned OTUs to several *Ascomycota sp*. (100% identity, *e* -value = 2e-87). For the other OTUs, the BLAST search narrowed the identity of the *Tylospora sp*. to *Tylospora asterophora* (99% identity, *e* -value = 1e91, accession: JQ711985.1), the *Thelephoraceae* member to a *Tomentella sp*. (97% identity, e-value = 2e90, accession: MH248057.1), and the *Leotiomycetes* member to the *Helotiaceae* family (100% identity, *e* -value = 2e80 for several *Meliniomyces* and *Helotiaceae* species). One unassigned OTU could not be identified with any certainty and was disregarded. Generally, fungal OTUs were less abundant in areas of moderate/high SRC influence than in those of low influence. *Tylospora asterophora*, *Tomentella sp*., and the member of the *Helotiaceae* were more abundant in the low influence sites than in moderate sites; counts of these OTUs and an *Ascomycota* sp. were also higher in the low influence sites than in the high influence sites. Between the moderate and the high influence sites, the *Ascomycota sp*. was more abundant in the former, whereas the *Tomentella sp*. was more abundant in the latter.

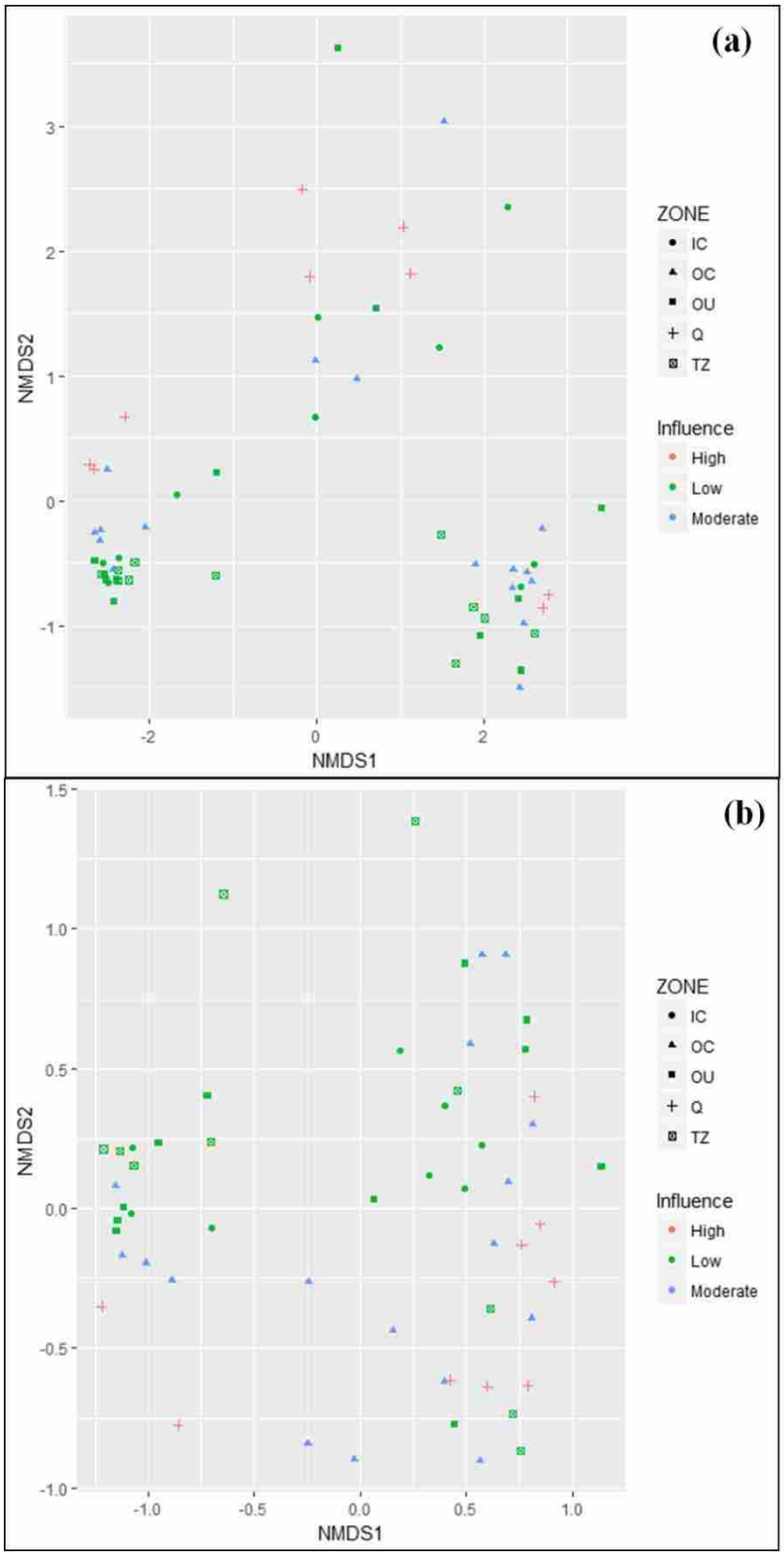


Figure 3.5: Non-metric multidimensional scaling (NMDS) biplots of 16S bacterial (a) and ITS fungal (b) community data for all horizons pooled together. Samples are colour-coded by Spanish River Carbonatite (SRC) influence level. IC, inner core; OC, outer core; OU, outside of the deposit; Q, quarry; TZ, transition zone.

Table 3.2: Bacterial OTUs of differential abundance between high and low/negligible SRC sites.

Phylum	Class	Order	Family	Genus	Abundance with SRC
Actinobacteria	Thermophilia	Gaiellales	Gaiellaceae	NA	Increased
Actinobacteria	Actinobacteria	Actinobacteriales	Micrococcaceae	NA	Increased
AD3	ABS-6	NA	NA	NA	Decreased
Acidobacteria	DA052	Ellin6513	NA	NA	Decreased
Acidobacteria	DA052	Ellin6513	NA	NA	Decreased
Acidobacteria	DA052	Ellin6513	NA	NA	Decreased
Acidobacteria	DA052	Ellin6513	NA	NA	Decreased
Acidobacteria	DA052	Ellin6513	NA	NA	Decreased
Acidobacteria	DA052	Ellin6513	NA	NA	Decreased
Acidobacteria	DA052	Ellin6513	NA	NA	Decreased
Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	NA	Decreased
Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	NA	Decreased
Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae	NA	Decreased
Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae	NA	Decreased
Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae	NA	Decreased
Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae	NA	Decreased
Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteriaceae	NA	Decreased
Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteriaceae	NA	Decreased
Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteriaceae	NA	Decreased
Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	Decreased
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobium	Bradyrhizobium sp.	Decreased
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiacae	Burkholderia bryophila	Decreased
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA	Decreased
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA	Decreased
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	NA	Decreased
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	NA	Decreased
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	NA	Decreased
Proteobacteria	Gammaproteobacteria	Xanthomonodales	Sinobacteriaceae	NA	Decreased

Discussion

This is the first comprehensive study investigating how the soil properties associated with a natural carbonatite deposit influence the plant and soil microbial communities in a Great Lakes–St. Lawrence forest ecosystem. As with most of this part of North America, the deposit is covered by a layer of overburden from past glaciation; however, despite this it was found that some areas above the deposit had an identifiable soil chemical signature indicative of SRC. The variable soil chemistry resulted in differences in plant community structure. This was demonstrated in areas of moderate SRC influence that had different plant species than those areas of low/negligible influence. However, the influence of SRC did not appear to be a dominant factor in shaping soil microbial communities. Nevertheless, several bacterial and fungal OTUs were found to be differentially abundant between areas of high and low/negligible SRC influence.

SRC, soils, and the influence of the deposit on the overlying ecosystem

Several factors indicated an influence of the SRC deposit on soil chemistry, as revealed by a comparison between zones. Because CEC, Ca, Al, S, Mn, and pH values of the OC soils (moderate influence) were often more similar to those of the exposed SRC comprising the Q samples (high influence) than to those of other zones (low/negligible influence), the increases in these parameters were considered to be a signature of SRC. The primary mineral of SRC is calcite, and like many other carbonatite deposits it is accompanied by a variety of nutrient-bearing accessory minerals such as biotite and apatite (Sage 1987). Despite there being an overburden layer of variable depth (0 m at the quarry to at least 56 m elsewhere) covering the deposit, it appears that these minerals are affecting some of the sampled soils. Kunzendorf and Secher (1987) made a similar suggestion for the Qaqarssuk carbonatite deposit in Greenland, which is covered by soils formed from alluvial deposits that are enriched in Nb and P originating from the underlying carbonatite. Thus, it can be inferred that in some areas the depth of the overburden is shallow enough that weathered SRC exerts an effect on the soils. This needs to be validated as there is not a reliable overburden map for the whole deposit. It is assumed that weathering of SRC fragments and their incorporation into the soils is responsible for the SRC signature identified in the OC sites surveyed here. This assumption is justified based on the similar soil chemistry between OC and Q sites, and by previous works demonstrating carbonatite influence on soil chemistries (e.g., Kunzendorf and Secher 1987; Vestin et al. 2006; Ahn et al. 2014). However, this study has not verified the presence of carbonatite minerals in the soil outside of the quarry (Q). Although the soil chemistry of the OC sites was similar to that of the Q, many values were significantly lower in the OC and it is likely that the overburden present in the OC dilutes the SRC influence. Additionally, topography, hydrology, and vegetation presumably play important roles in the

transfer of carbonatite elements into the glacial till soil. For instance, where the deposit is near the surface at higher elevations, a signature of SRC might be found both in soils at these locations and in nearby topographical lows where downward water movement through the soils would have transported some minerals or elements. Nutrient translocation by the action of plants could also have occurred and a mechanism for this is provided by the nutrient-uplifting hypothesis of Jobbágy and Jackson (2004), which posits that trees (and other vegetation) are active in transporting lithosphere-derived nutrients to the surface of soils. This translocation occurs when lithosphere nutrients are incorporated into above- and below-ground plant biomass, and subsequently released when the plant organic matter decomposes close to the soil surface.

The soil chemistry differences above the deposit, e.g., in pH or Mn content, indicate that in some areas SRC has had a quantifiable influence on the soils. Above-ground differences in plant community composition are, therefore, likely linked to changes in soil chemistry. Several plant species unique to the moderate influence sites (i.e., the OC) are typically those colonizing areas post-disturbance or that are tolerant to alkaline soil pH. For instance, *C. peregrina* has been considered a weed, and post-fire disturbance conditions are favourable for its growth (Hall et al. 1976). As none of the outer core sites displayed signs of recent disturbance (e.g., being dominated by younger trees or displaying signs of fire damage on older wood), it is likely that the abundance of ruderal species in the OC is not due to a disturbance event but to the presence of SRC. With the large differences in soil pH between areas of moderate and of low/negligible SRC influence, it appears that one of the main effects of the deposit has been the creation of ecological microhabitats ("islands") of more basic soil pH in what would otherwise be a large surrounding area ("sea") of more acidic soils. The more basic the soils, the greater likelihood they will be colonized by species tolerant of soil pH changes, i.e., ruderal species. Based on the Shannon diversity indices, where areas of moderate influence were seen to have lower indices than those areas without SRC influence, we hypothesize that the altered soil chemistry caused by SRC constrains the abundance and diversity of the plant species present in these areas. The data collected regarding tree species show the same trend whereby in moderate influence sites there were fewer tree species and fewer individuals than in low/negligible sites (Table S3.3). This might be due to the soil chemistry restricting the types and number of plant species and (or) the spatial separation from areas where more alkaline-adapted species are present and could serve as seed sources. These species could also be less tolerant of the higher, potentially toxic Al concentrations found in the more acidic soil conditions. A similar situation whereby the bedrock and soil chemistry controlled the distribution of plant species was found in a broad survey of rock habitat vegetation performed by Tyler (1996). To verify this hypothesis, the soil pH across the deposit area could be assessed, and the areas of higher pH (and thus presumably under carbonatite influence) identified and surveyed for vegetation community composition. Such a study would also assist in determining the

magnitude of overburden depth and hydrology/topography in expressing the effects of the carbonatite on the deposit area soils.

Although it was expected that the soil microbial communities would be significantly shaped by differences in pH or by the abundance of elements provided by the weathering of SRC's nutrient bearing minerals, the data did not support this hypothesis. For instance, many species of bacteria that have been associated with the weathering of carbonatite minerals, e.g., *Burkholderia* (Uroz et al. 2011) or *Pseudomonas* (Colin et al. 2017), were not more abundant in the moderate or high influence sites than in the low/negligible sites. The only bacterial OTU consistently in higher abundance in SRC affected areas was tentatively identified as a member of the genus *Arthrobacter* belonging to the family Micrococcaceae. Members of this genus have been shown to utilize a diverse set of carbon/nitrogen sources (Hagedorn and Holt 1975). Furthermore, their ability to metabolize aliphatic amino acids and aromatic hydrocarbons (Hagedorn and Holt 1975), as well as their nitrification activity, were found to be higher at more neutral than acidic pHs (Brierly and Wood 2001). The ability to utilize a variety of nutritional substrates and to nitrify under the more basic soil conditions in SRC-affected areas would be beneficial to this microbial group as SRC would not provide N, and these characteristics could explain the higher presence of *Arthrobacter* in areas with the SRC signature. This would be especially important in the Q, where the soils almost exclusively consist of SRC particles. In terms of the fungal OTUs, most were found to decrease in abundance with increasing SRC influence. This is in agreement with the observations made by Rousk et al. (2010) that fungi tend to dominate in acidic soils whereas bacteria prefer more neutral soils.

The most abundant bacterial families detected in our soil samples were either saprotrophic and (or) associated with the rhizosphere of plants (Table S3.4). Similarly, aside from the *Pseudeurotiaceae*, most fungal families were saprotrophic and (or) ectomycorrhizal (Table S3.4). The identity of tree species dominant in a given area can be a strong determinant of fungal community composition (Urbanová et al. 2015). As such, differences in leaf litter composition and (or) mycorrhizal hosts may provide an explanation for the differences seen in fungal abundances between low/negligible and the high or moderate SRC influence soils. Additionally, increasing the pH to values similar to those observed in the moderately-influenced sites has previously been reported to negatively affect the survival of ectomycorrhizae on *P. abies* roots (Lehto 1994). This suggests that the increased soil pH resulting from the presence of SRC may not be conducive to ectomycorrhizal colonization of the tree species at the deposit. Given our focus in this study on the assessment of the colonization of herbaceous plants by arbuscular mycorrhizal fungi, the colonization of trees by ectomycorrhizal fungi needs to be investigated in future studies. Furthermore, the functional classification of the microorganisms presented here was very general, and exploration of differences in metabolic diversity versus taxonomic diversity may yield further insight into microbial responses to

carbonatite. Finally, it is notable that members of the Archaea did not arise during the taxonomic classification; whether this is due to actual differences in abundance or a result of the DNA sequencing approach used here is unknown.

Conclusion

The results reported here are the first that show that a carbonatite deposit can contribute to shaping the overlying ecosystem. We detected a SRC signature in some soils, which had an effect on both the plant and soil microbial communities. Furthermore, using Illumina Mi-Seq, we were able to investigate both community-level and individual OTU-level responses of microbes to SRC in the deposit. Overall, it appears that when the overburden is shallow enough to allow for the carbonatite deposit to influence the soil chemistry, pockets of more basic soils have developed in an area of otherwise predominantly acidic soils. In turn, this has constrained the abundance and diversity of plant species in these areas and affected the abundance of several microorganisms. However, most groups of bacteria and fungi in communities were unaffected by the SRC. Based on these findings, in areas where carbonatite deposits are found close to the surface, it can be expected that ecological effects may emerge, which may or may not lead to unique ecosystem-level features. As such, we recommend that ecosystems in and around these deposits should be studied using similar approaches to those reported here to better understand these sites and, if needed, develop appropriate strategies for environmental protection should these areas be targeted for mining. Finally, although the soil properties, plants, and soil microorganisms were emphasized here, impacts on other relevant organisms (e.g., insects or other fauna) should be included in future work.

Acknowledgements

The authors wish to give thanks to Lisa Derickx, Meagan Sutherland, and Jennifer Bird for their assistance in the field, and to the anonymous reviewers for their comments for strengthening the manuscript. Funding was provided by Ontario Genomics for DNA sequencing, and from an NSERC ENGAGE grant in partnership with Boreal Agrominerals Inc. awarded to PMA.

References

Ahn JS, Youm S-J, Cho Y-C, Shin S-C, and Cho W-H. 2014. Geochemical survey of rare earth elements (REEs) in the concealed ore body of Hongcheon, Korea. Environmental Earth Sciences, 72: 2153–2161. DOI: 10.1007/s12665-014-3123-y

Bokulich NA, and Mills DA. 2013. Improved selection of internal transcribed spacer-specific primers enables quantitative, ultra-high-throughput profiling of fungal communities. Applied and Environmental Microbiology, 79: 2519–2526. PMID: 23377949 DOI: 10.1128/AEM.03870-12

Brierly EDR, and Wood M. 2001. Heterotrophic nitrification in an acid forest soil: isolation and characterisation of a nitrifying bacterium. Soil Biology and Biochemistry, 33: 1403–1409. DOI: 10.1016/S0038-0717(01)00045-1

Brooks RR. 1972. Geobotany and biogeochemistry in mineral exploration. Harper and Row, Publishers, Inc., New York, New York. 290 p.

Brundrett M, Bougher N, Dell B, Grove T, and Malajczuk N. 1996. Working with mycorrhizas in forestry and agriculture. Australian Centre for International Agricultural Research Monograph 32. Australian Centre for International Agricultural Research, Canberra, Australia. 374 p.

Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, and Knight R. 2010. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics, 26: 266–267. PMID: 19914921 DOI: 10.1093/bioinformatics/btp636

Colin Y, Nicolitch O, Turpault M-P, and Uroz S. 2017. Mineral types and tree species determine the functional and taxonomic structures of forest soil bacterial communities. Applied and Environmental Microbiology, 83: e02684-16. PMID: 28003192 DOI: 10.1128/AEM.02684-16

Dickinson R, Dickinson T, and Metsger D. 2004. ROM field guide to wildflowers of Ontario. McClelland & Stewart, Toronto, Ontario. 416 p.

Dyke AS, Andrews JT, Clark PU, England JH, Miller GH, Shaw J, et al. 2002. The Laurentide and Innuitian ice sheets during the Last Glacial Maximum. Quaternary Science Reviews, 21: 9–31. DOI: 10.1016/S0277-3791(01)00095-6

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32: 1792–1797. PMID: 15034147 DOI: 10.1093/nar/gkh340

Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics, 26: 2460–2461. PMID: 20709691 DOI: 10.1093/bioinformatics/btq461

Edgar RC. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods, 10: 996–998. PMID: 23955772 DOI: 10.1038/nmeth.2604

Fowler J, Cohen L, and Jarvis P. 1998. Practical statistics for field ecology. 2nd edition. John Wiley & Sons Ltd., Chichester, UK. 272 p.

Hagedorn C, and Holt JG. 1975. A nutritional and taxonomic survey of *Arthrobacter* soil isolates. Canadian Journal of Microbiology, 21: 353–361. PMID: 1116046 DOI: 10.1139/m75-050

Hall IV, Aalders LE, and Everett CF. 1976. The biology of Canadian weeds: 16. *Comptonia peregrina* (L.) Coult. Canadian Journal of Plant Science, 56: 147–156. DOI: 10.4141/cjps76-022

Hanslinger E, Ottner F, and Lundström US. 2007. Pedogenesis in the Alnö carbonatite complex, Sweden. Geoderma, 142: 127–135. DOI: 10.1016/j.geoderma.2007.08.014.

Heinrich EWM. 1980. The geology of carbonatites. Rand McNally and Company, Huntington, West Virginia. 601 p.

Jobbágy EG, and Jackson RB. 2004. The uplift of soil nutrients by plants: biogeochemical consequences across scales. Ecology, 85: 2380–2389. DOI: 10.1890/03-0245

Jones AP, Genge M, and Carmody L. 2013. Carbonate melts and carbonatites. Reviews in Mineralogy and Geochemistry, 75: 289–322. DOI: 10.2138/rmg.2013.75.10

Jones JMC. 2016. Spanish River Carbonatite: its benefits and potential use as a soil supplement in agriculture. M.Sc. thesis, Wilfrid Laurier University, Waterloo, Ontario. 110 p.

Kunzendorf H, and Secher K. 1987. Dispersion of niobium and phosphorus in soil overlying the Qaqarssuk Carbonatite Complex, Southern West Greenland. Journal of Geochemical Exploration, 28: 285–296. DOI: 10.1016/0375-6742(87)90053-7

Lehto T. 1994. Effects of soil pH and calcium on mycorrhizas of *Picea abies*. Plant and Soil, 163: 69–75. DOI: 10.1007/BF00033942

Love MI, Huber W, and Anders S. 2014. Moderated estimation of fold change and dispersion for RNAseq data with DESeq2. Genome Biology, 15: 550. PMID: 25516281 DOI: 10.1186/s13059-014-0550-8

Mariano AN. 1989. Nature of economic mineralization in carbonatites and related rocks. In Carbonatites: genesis and evolution. Edited by K Bell. Unwin Hyman, London, UK. pp. 149–172.

Masella AP, Bartram AK, Truszkowsi JM, Brown DG, and Neufeld JD. 2012. PANDAseq: paired-end assembler for illumina sequences. BMC Bioinformatics, 13: 31. PMID: 22333067 DOI: 10.1186/1471-2105-13-31

McGonigle TP, Miller MH, Evans DG, Fairchild GL, and Swan JA. 1990. A new method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. New Phytologist, 115: 495–501. DOI: 10.1111/j.1469-8137.1990.tb00476.x

Moon CJ. 2006. Exploration geochemistry. *In* Introduction to mineral exploration. 2nd edition. *Edited by* CJ Moon, MKG Whateley, and AM Evans. Blackwell Publishing, Malden, Massachusetts. pp. 155–178.

Myrvang MB, Hillersøy MH, Heim M, Bleken MA, and Gjengedal E. 2016. Uptake of macro nutrients, barium, and strontium by vegetation from mineral soils on carbonatite and pyroxenite bedrock at the Lillebukt Alkaline Complex on Stjernøy, Northern Norway. Journal of Plant Nutrition and Soil Science, 179: 705–716. DOI: 10.1002/jpln.201600328

Newcomb L. 1989. Newcomb's wildflower guide. Little, Brown and Company, Boston, Massachusetts. 490 p.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. 2016. Multivariate analysis of ecological communities in R: vegan tutorial. R package version, 1.7 [online]: Available from cran.r-project.org/web/packages/vegan/vegan.pdf.

Petrus JA, Ames DE, and Kamber BS. 2015. On the track of the elusive Sudbury impact: evidence for a chondrite or comet bolide. Terra Nova 27: 9-20. DOI: 10.1111/ter.12125.

Price MN, Dehal PS, and Arkin AP. 2010. FastTree 2—approximately maximum-likelihood trees for large alignments. PLoS ONE, 5: e9490. PMID: 20224823 DOI: 10.1371/journal.pone.0009490

R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria [online]: Available from R-project.org/.

Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, et al. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal, 4: 1340–1351. PMID: 20445636 DOI: 10.1038/ismej.2010.58

Sage RP. 1987. Geology of carbonatite–alkalic rock complexes in Ontario—Spanish River Carbonatite Complex—District of Sudbury. Ministry of Northern Development and Mines, Toronto, Ontario. ISBN: 0-7729-0565-7 [online]: Available from geologyontario.mndmf.gov.on.ca/mndmfiles/pub/data/imaging/S030/S030.pdf.

Stohlgren TJ, Falkner MB, and Schell LD. 1995. A modified-Whittaker nested vegetation sampling method. Vegetatio, 117: 113–121. DOI: 10.1007/BF00045503

Takahashi S, Tomita J, Nishioka K, Hisada T, and Nishijima M. 2014. Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. PLoS ONE, 9: e105592. PMID: 25144201 DOI: 10.1371/journal.pone.0105592

Talvitie J. 1979. Remote sensing and geobotanical prospecting in Finland. Bulletin of the Geological Society of Finland, 51: 63–73. DOI: 10.17741/bgsf/51.1-2.007

Tyler G. 1996. Soil chemistry and plant distributions in rock habitats of southern Sweden. Nordic Journal of Botany, 16: 609–635. DOI: 10.1111/j.1756-1051.1996.tb00279.x

Urbanová M, Šnajdr J, and Baldrian P. 2015. Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. Soil Biology and Biochemistry, 84: 53–64. DOI: 10.1016/j.soilbio.2015.02.011

Uroz S, Oger P, Lepleux C, Collignon C, Frey-Klett P, and Turpault M-P. 2011. Bacterial weathering and its contribution to nutrient cycling in temperate forest ecosystems. Research in Microbiology, 162: 820–831. PMID: 21315149 DOI: 10.1016/j.resmic.2011.01.013

Vestin JLK, Nambu K, van Hees PAW, Bylund D, and Lundström US. 2006. The influence of alkaline and non-alkaline parent material on soil chemistry. Geoderma, 135: 97–106. DOI: 10.1016/j.

Vestin JLK, Söderberg U, Bylund D, Nambu K, van Hees PAW, Haslinger E, et al. 2013. The influence of alkaline and non-alkaline parent material on Norway spruce tree chemical composition and growth rate. Plant and Soil, 370: 103–113. DOI: 10.1007/s11104-013-1615-2

Wang B, and Qiu Y-L. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza, 16: 299–363. PMID: 16845554 DOI: 10.1007/s00572-005-0033-6

Wang Q, Garrity GM, Tiedje JM, and Cole JR. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology, 73: 5261–5267. PMID: 17586664 DOI: 10.1128/AEM.00062-07

Woolley AR, and Kempe DRC. 1989. Carbonatites: nomenclature, average chemical compositions, and element distribution. In Carbonatites: genesis and evolution. Edited by K Bell. Unwin Hyman, London, UK. pp. 1–14.

Woolley AR, and Kjarsgaard BA. 2008a. Paragenetic types of carbonatite as indicated by the diversity and relative abundances of associated silicate rocks: evidence from a global database. The Canadian Mineralogist, 46: 741–752. DOI: 10.3749/canmin.46.4.741

Woolley AR, and Kjarsgaard BA. 2008b. Carbonatite occurrences of the world: map and database. Geological Survey of Canada Open File Report No. 5796 [online]: Available from geoscan.nrcan.gc.ca/starweb/geoscan/servlet.starweb?path=geoscan/fulle.web&search1=R=225115.

Chapter 3 - Supplemental data

Supplemental Table 3.1: Mean soil chemistry parameters for horizons across each zone. Values are means of 1-4 per horizon/zone.

	Quarry		Inner Core			Outer Core		
Horizons	**A**	**B**	**A**	**B**	**C**	**A**	**B**	**C**
Depth (cm)	23.8	23.5	14.0	13.7	42.5	22.3	23.3	24.0
pH	7.6	7.6	4.8	4.8	4.8	5.0	5.3	5.5
OM (%)	0.9	1.2	0.8	1.4	2.4	1.3	0.6	0.3
P (ppm)	20.0	39.0	52.5	39.0	47.0	NA	83.0	16.0
K (ppm)	35.3	36.5	34.5	55.3	39.7	44.0	33.0	36.0
Ca (ppm)	7694.8	8176.0	179.5	108.3	213.8	414.0	214.0	184.0
Mg (ppm)	33.0	27.7	19.5	17.8	19.5	27.0	28.0	24.0
S (ppm)	7.3	11.0	20.5	23.3	13.3	14.0	8.0	6.0
Mn (ppm)	59.9	59.1	3.7	7.3	9.9	38.0	13.1	9.0
Fe (ppm)	197.7	690.3	122.1	98.1	120.2	278.2	113.4	66.3
Al (ppm)	786.8	690.3	1997.0	1895.8	1508.3	1664.0	1168.0	1277.0
CEC meq/100g	39.1	41.5	3.6	4.0	7.0	5.0	2.4	2.2
TN (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

	Transition Zone			Outside Deposit		
Horizons	**A**	**B**	**C**	**A**	**B**	**C**
Depth (cm)	10.5	19.5	39.5	9.8	16.0	21.8
pH	4.7	4.9	5.0	4.9	4.9	4.9
OM (%)	5.2	2.0	0.5	5.5	2.1	1.0
P (ppm)	24.3	32.5	58.3	23.8	21.3	48.8
K (ppm)	37.5	29.0	NA	39.7	37.5	62.5
Ca (ppm)	158.5	123.5	152.0	320.5	262.3	171.0
Mg (ppm)	20.5	17.5	15.0	29.5	24.0	21.0
S (ppm)	32.5	39.5	32.7	26.0	44.8	26.5
Mn (ppm)	9.6	6.6	4.0	20.8	5.6	4.9
Fe (ppm)	357.0	154.6	65.9	344.0	120.0	74.3
Al (ppm)	2094.0	1993.0	1779.0	1964.3	2102.3	1925.3
CEC meq/100g	11.3	5.6	2.5	10.4	6.7	4.0
TN (%)	0.2	0.1	0.1	0.2	0.1	0.1

Supplemental Table 3.2: Arbuscular mycorrhizal colonization of most abundant species by deposit zone. Where possible, the top three most abundant species per site were assessed for mycorrhizal colonization. The percentage of hyphal (HypCol, number of hyphae/total fungal structures), arbuscular (ArbCol, number of arbuscules/total fungal structures), and vesicular (VesCol, number of vesicles/total fungal structures) colonization is given per species, along with the SRC influence category (Inf.).

Zone	Site #	Inf.	Species	ArbCol	HypCol	VesCol
Q	7	High	*Conyza canadensis*	0.03	0.56	0.37
Q	7	High	*Agrostis gigantea*	0.04	0.21	0.70
Q	7	High	*Anaphalis margaritacea*	0.31	0.29	0.27
Q	12	High	*Conyza canadensis*	0.08	0.30	0.41
Q	12	High	*Poa compressa*	0.15	0.24	0.38
Q	12	High	*Matricaria discoidea*	0.06	0.41	0.09
IC	3	Low	*Geum fragarioides*	0.07	0.31	0.12
IC	3	Low	*Maianthemum canadense*	0.03	0.57	0.18
IC	3	Low	*Eurybia macrophylla*	0.00	0.27	0.05
IC	4	Low	*Cornus canadensis*	0.06	0.49	0.06
IC	4	Low	*Maianthemum canadense*	0.01	0.35	0.12
OC	6	Moderate	*Solidago altissima*	0.09	0.49	0.02
CO	6	Moderate	*Pilosella officinarum*	0.18	0.57	0.15
CO	10	Moderate	*Diervilla lonicera*	0.08	0.54	0.30
CO	10	Moderate	*Eurybia macrophylla*	0.01	0.57	0.04
TZ	17	Low	*Cornus canadensis*	0.01	0.44	0.06
TZ	17	Low	*Maianthemum canadense*	0.01	0.44	0.05
TZ	18	Low	*Maianthemum canadense*	0.17	0.40	0.06
OU	15	Low	*Geum fragarioides*	0.03	0.54	0.04
OU	15	Low	*Maianthemum canadense*	0.01	0.42	0.20
OU	15	Low	*Eurybia macrophylla*	0.01	0.80	0.03
OU	19	Low	*Cornus canadensis*	0.01	0.50	0.01
OU	19	Low	*Maianthemum canadense*	0.01	0.51	0.07

Supplemental Table 3.3: Cumulative number of individual mature trees counted in the modified-Whittaker plots for each zone (a) or at each influence level (b). Numbers are the sum of trees per zone from each of the two sites, with exceptions for the influence categories*. Abbreviations: IC = inner core, TZ = transition zone, OC = outer core, OU = outside deposit.

a)

Species	IC	OC	TZ	OU
Pinus banksiana	266	0	102	19
Pinus resinosa	55	0	0	109
Pinus strobus	0	4	6	9
Picea glauca	1	11	27	2
Abies balsamea	2	9	19	40
Populus tremuloides	27	68	3	11
Betula papyrifera	0	2	18	29

b)

Species	Low	Moderate
Pinus banksiana	129	0
Pinus resinosa	55	0
Pinus strobus	5	6
Picea glauca	10	27
Abies balsamea	20	19
Populus tremuloides	14	3
Betula papyrifera	16	18

*total individuals per area/sites per influence level (n= 3 sites for low, 1 for moderate)

Supplemental Table 3.4: Some known functional roles for relevant taxonomic groups in this study. Each functional group (Family/Genus) is addressed separately for bacteria and fungi. Abbreviated references are provided below the table.

Bacteria (Family-level)	**Function(s)**	**Ref.**
Bradyrhizobiaceae	Crop rhizosphere, saprotrophic	3, 4, 5, 9
Comamonadaceae	Crop rhizosphere, saprotrophic	3, 4
Enterobacteriaceae	Crop rhizosphere, saprotrophic	4, 8
Hyphomicrobiaceae	Saprotrophic	8
Micrococcaceae	Crop rhizosphere, saprotrophic	4, 8
Pseudomonadaceae	Crop rhizosphere, role in plant immune function	7
Sinobacteraceae	Saprotrophic	10, 11
Fungi (Family-level)		
Atheliaceae	Ectomycorrhizal, parasitic, saprotrophic, pathogenic	13, 14, 15
Cortinariaceae	Ectomycorrhizal	16
Hygrophoraceae	Ectomycorrhizal, parasitic	17
Pseudeurotiaceae	Pathogen, saprotrophic	18, 19
Russulaceae	Ectomycorrhizal	16
Thelephoraceae	Ectomycorrhizal	2, 16
Bacteria (Genus-level)		
Arthrobacter	Saprotrophic, phosphate-solubilizer, produces indoleacetic acid	5, 6
Pseudoarthrobacter	Temperate soil microbe with bioremediation potential	12
Fungi (Genus-level)		
Tylospora asterophora	Ectomycorrhizal, saprotrophic	1
Tomentella sp.	Ectomycorrhizal	2

References: **1** - Eberhardt et al. 1999. Can J Bot 77: 11-21., **2** - Lilleskov et al. 2004. Mycologia 97: 762-769., **3** - Folman et al. 2008. FEMS Microbiol Ecol 63: 181-191., **4** - Haichar et al. 2008. The ISME Journal 2: 1221-1230., **5** - Negassa et al. 2015. PLoS One 10: e0123999., **6** - Bello-Akinosho et al. 2016. Biomed Research International vol. 2016., **7** - Neal and Ton. 2013. Plant Signal Behav 8: e22655, **8** - Zhou et al. 2016. Sci Rep 6: 22186., **9** - Zarraonaindia et al. 2015. mBio 6: e02527-14, **10** - Eichorst and Kuske. 2012. Appl and Env Microbiol 78: 2316-2327., **11** - Pershina et al. 2015. PLoS One 10: e0145072., **12** - Backman et al. 2004. Appl Environ Microbiol 70:2952-2958., **13** - Shi et al. 2002. Mycorrhiza 12: 303-311., **14** - Yurchenko et al. 2003. Mycological Progress 4: 275-284., **15** - Matheny et al. 2006. Mycologica 98: 982-995., **16** - Trocha et al. 2012. Mycorrhiza 22: 121-134., **17** - Lodge et al. 2014. Fungal Diversity 64: 1-99., **18** - Reynolds and Barton. 2014. PLoS One 9: e86437., **19** - Malloch and Cain. 1970. Can. J. Bot. 48: 1815-1825.

Chapter 4 - Carbonatite enhances growth and nutrition of plants via their nutrient-foraging strategy

This chapter will be submitted as a research paper in the journal Soil Biology and Biochemistry. Currently, it is written as a thesis chapter and contains more data than would normally be present in a manuscript. The final paper is intended to provide a qualitative assessment of the effect of SRC on two crop plants, and show a mechanism of action to explain why those effects occur.

Author contributions:

JMCJ, PMA, and FCG conceived and designed the study. JMCJ performed the experiments/collected the data. JMCJ, PMA, and FCG analyzed and interpreted the data. PMA and FCG contributed resources. JMCJ, PMA, and FCG drafted or revised the manuscript.

Carbonatite enhances growth and nutrition of plants via their nutrient foraging strategy - a test with wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.)

Jones JMC[1], Antunes PM[2] and Guinel, FC[1]

1 – Biology Department, Wilfrid Laurier University, Waterloo, Ontario, Canada

2 – Biology Department, Algoma University, Sault Ste. Marie, Ontario, Canada

Abstract:

There is renewed interest in exploiting unprocessed rocks and minerals for agriculture to meet sustainability challenges. Carbonatites are of particular relevance as rock fertilizers because of their rapid weathering rates and nutrient-bearing accessory minerals. Although previous work with Spanish River Carbonatite (SRC) and pea demonstrated benefits for plant growth and nodulation, it was unclear to what extent SRC was responsible. Here, our goals were to compare the action of SRC to that of lime, to extend the study to another plant species (wheat), and to determine a mechanism by which SRC imparts its effects. Both pea and wheat were grown under controlled conditions in a low-nutrient substrate, and a variety of plant and soil microbial responses were measured. Wheat showed a strong positive response to SRC amendment. This response was markedly different from that of lime, and was modulated by its nutrient foraging strategy via changes in root architecture. The response of pea to SRC was complex, and seemed to be a function of carbonatite-microorganism interactions. Notably, microorganisms were also affected by SRC, which had a selective effect on their abundance (with pea) and activity (with wheat). These results demonstrate that while carbonatite rock fertilizers can positively influence plants, their benefits depend on the strategy by which the plants acquire nutrients from the soil. Further work is needed to characterize how carbonatites interact with different plant species, and how benefits can be realized under field conditions, e.g., which soils or management practices are most conducive to rock weathering.

Introduction:

A primary goal of agriculture is the efficient and sustainable delivery of nutrients to plants to produce viable crop yields. One tool to help achieve this goal is rock fertilizers; these are geological resources chosen for their elemental composition and capacity to serve as nutrient sources for plants (van Straaten 2007). One of the primary benefits of such materials is the provision of slow-release, on-demand nutrients to plants, and much of the interest in rock fertilizers is based around their usefulness as cheap, locally-sourced resources (van Straaten 2007).

Among the different rock types, carbonatites have some of the most rapid weathering rates because of their mineral composition. These rocks are defined by having ≥ 50% carbonate minerals (Woolley and Kempe 1989) and minor amounts of silicates (Jones et al. 2013). The most common carbonatites are composed of calcium, magnesium, or sometimes iron carbonates (Woolley and Kempe 1989). Furthermore, carbonatite deposits have a high diversity of associated minerals, and often contain apatite or biotite (Woolley and Kempe 1989). As such, carbonatites are of particular interest for agricultural purposes: in theory they could serve as multi-nutrient slow-release fertilizers. Additionally, the high Ca and Mg content of carbonatites means that they could also act as liming agents to counter soil acidification (Myrvang et al. 2016). Despite this, carbonatites have been minimally explored for their agricultural potential, and the research has typically focused only on plant nutrition and biomass.

One of the largest problems hindering the effectiveness of rock fertilizers as meaningful nutrient sources is that they may not weather rapidly enough to provide sufficient nutrients to justify their application (Bakken et al. 1997a; Reddy et al. 2002). Mineral weathering is a complex process that depends on the mineral's composition (e.g., silica content), the climate (e.g., temperature and moisture), the soil pH, and the influence of organisms such as plants and microbes (Goldich 1938; Uroz et al. 2009; Ahmed and Holmström 2015). It is expected that the effectiveness of rock fertilizers will therefore be the highest under conditions where weathering is promoted.

Evidence for the impact of soil pH has already been shown in the literature. A Canadian study by Arcand et al. (2010) used two locally-sourced igneous rocks and one imported sedimentary rock with the aim of assessing their effectiveness to serve as P-sources under field conditions. The intent was to utilize the capacity of buckwheat, a plant which acidifies its rhizosphere, to promote weathering of the rocks and subsequent uptake of P. Once taken up, the P-enriched buckwheat could then be used as a source of P for future crops. The two local phosphate rocks were obtained from the Kapuskasing and Spanish River carbonatite (SRC) deposits in Northern Ontario, Canada. Overall, the authors concluded that high soil pH and Ca levels were not conducive to mineral weathering, and only the sedimentary phosphate rock at the highest application rate (800 kg P ha^{-1}) was effective at P delivery. In two parallel studies, Bakken *et al.* investigated a variety of rocks and minerals, including carbonatite from the Lillebukt deposit in Stjernøy, Norway, and their effects on barley (Bakken et al. 1997a) and ryegrass (Bakken et al. 1997b). The overarching goal was to find alternative to chemical K fertilizers to minimize the antagonistic effects high soil K volumes can have on Mg and Ca uptake by plants. In these studies, the rocks and minerals were tested for their ability to provide K to plants and were compared against water-soluble KCl fertilizer. Both barley and ryegrass were able to access K from the carbonatite to a similar extent as from KCl (Bakken et al. 1997a, 1997b), and the carbonatites were more effective in this capacity than the non-carbonatite gneiss and schists. The contradictory findings of these studies highlight the importance of context to carbonatite usefulness: effective K delivery from carbonatites was found when used in acidic soils (Bakken et al. 1997a, 1997b) but carbonatites were ineffective at supplying P in more basic soils (Arcand et al. 2010). There is evidence for carbonatites to serve as effective plant nutrient sources; however, the optimal conditions for their weathering, their effect on plant growth, and their interactions with soil microorganisms remain to be defined.

Here, a multi-faceted approach is taken to investigate the effects of SRC on two crop plants and its interaction with microorganisms. The SRC deposit, about 3.25 km^2 in diameter, is located near Sudbury, ON, Canada, and has a relatively well-defined mineralogy (Sage 1987; Jones et al. 2019). Because it is composed of a number of nutrient-bearing minerals (e.g., calcite, biotite, apatite), SRC

has been used locally by farmers for several years as a commercial soil amendment for various crops (e.g., asparagus, corn, soybean). A preliminary investigation of SRC in this capacity revealed that it has beneficial effects on the growth of pea (*Pisum sativum* L.), but it was unclear by which means these effects were being exerted (Jones 2016). Given the number of unknowns regarding the effectiveness of carbonatites and the possible factors influencing it, this investigation was conducted under controlled conditions using a low-carbon substrate mixture. The goal was to assess the usefulness of SRC as a rock fertilizer for crop plants when used under low nutrient conditions, and to define what effect it has on crop plant growth and on soil microorganism abundance and activity. Building upon previous work by Jones (2016), I tested the hypothesis that the effects of SRC on pea are due to changes in substrate pH, and that these effects can be reproduced with agricultural lime. The impact of SRC on wheat (*Triticum aestivum* L.) was also assessed. Wheat is an important crop plant in Canada, and as a monocot and non-legume plant, it may respond to SRC in a manner different from that of pea.

Common Materials and Methods:

Substrates and amendments

A low-nutrient 1:1 mixture of vermiculite:Turface® was used as a common substrate in all experiments to minimize the influence of soil nutrients and organic matter and to mimic the conditions under which rock fertilizers should be effective (i.e., a nutrient-depleted or acidic soil). Vermiculite is a micaceous mineral which contains magnesium, aluminum, and iron silicates (Slawek 2013). The grain size of the vermiculite used here was between 1 and 6 mm. Turface® is an artificial heat-treated silica clay (Plant Products Co. Ltd., Brampton, ON, Canada) containing aluminum and iron silicates and in this experiment it had a grain size of 0.8 - 3.4 mm (Profile Products 2015). Spanish River Carbonatite is mined from a deposit located near Sudbury in Northern Ontario, and is primarily composed of calcite, biotite, and apatite (Sage 1987). It is sold as a soil amendment in the form of a crushed rock product. It was obtained from Boreal Agrominerals Inc. (Brampton, ON,

Canada) in retail packages containing material between 0.3 - 2.0 mm grain size. The SRC was used exclusively in a 1:10 ratio with the substrate, as previous work found that this was the optimal ratio for plant growth and increased substrate pH (Jones 2016). Calcitic lime was obtained from Quality Fertilizers Inc. (Shakespeare, ON, Canada) with a grain size of approximately 2.5 mm. It was used only in a ratio of 1:15 lime:substrate to match the pH obtained with SRC. Amendments were homogenously mixed into the substrate and de-ionized water (or a nutrient solution, where indicated) was added to give a 20% (vol/vol) water content. The amended substrates used for pea were autoclaved at 121°C and 33 psi for 2 hours prior to planting to avoid introduction of microorganisms from the substrates or the amendments. With wheat, the experiments required substrate volumes too large to be autoclaved, and so only the amendments were sterilized to remove any amendment-specific microorganisms.

Microbial inoculants and inoculation

Overall, two separate microbial inoculations were utilised: an agricultural microbial mixture to provide an agriculturally-relevant soil microbial community, and a rhizobia solution to inoculate pea for nodulation studies. The agricultural microbial solution was prepared from a variety of agricultural soils with different crop types collected in Ontario, Canada (Table S4.1) which were stored at 4°C. These soils were pooled to minimize microorganism effects from any single source, and microorganisms were extracted by shaking a 1:9 soil:de-ionized water mixture at 125 rpm for 2h, discarding the soil, and retaining the liquid as inoculant (adapted from Lindahl and Bakken, 1995). The solution was made fresh (< 24 h before use) for every experiment and stored at 4°C prior to use. All wheat substrates were inoculated with 10mL of this solution around each seed upon planting. Where indicated, pea was also inoculated with this microbial homogenate. No colonization of roots by arbuscular-mycorrhizal fungi (AMF) was detected throughout the study, and so the inoculum was not considered to serve as a source of AMF propagules.

For the pea nodulation experiment, plants were inoculated at 4 days after planting (DAP) with 5 mL of a 5 % bacterial solution applied to the substrate around the emerged shoot (Guinel and Sloetjes 2000). The rhizobia, *Rhizobium leguminosarum* bv. *viciae* strain 128C53K, were cultured in a nitrogen-free yeast-mannitol broth (Vincent 1970) and incubated in an orbital-shaking water-bath (New Brunswick Scientific, Edison, NJ, USA) at 100 rpm and 25 °C until the bacterial population reached stationary growth (OD600 = 0.8-1.0).

Materials and Methods - Pea

Growth conditions

Seeds of pea (*Pisum sativum* cv. Sparkle) were surface-sterilized for ten minutes with an 8 % sodium hypochlorite solution and rinsed three times with sterile water (Guinel and Sloetjes 2000). Following imbibition (16-18 hours in the dark), they were individually planted in black Cone-tainers™ (656 mL volume; Stuewe and Sons, Tangent, OR, USA) and placed in trays filled with 1.5 L of de-ionized water; trays were separated by treatment group. Plants were watered as needed by adding 1.5 L de-ionized water to the tray and after 10 days, plants were provided with a modified Hoagland solution (Guinel and Sloetjes 2000) every third watering. When plants had been inoculated with rhizobia, they were instead given a reduced-nitrogen modified Hoagland solution (0.5 mM $Ca(NO_3)$) to minimize inhibition of nodulation. Plants were grown in the growth room facility at Wilfrid Laurier University (Waterloo, ON, Can) under a 16-hour day (23 °C) and 8-hour night (18 °C) photoperiod cycle. Light was supplied through high-pressure sodium, fluorescent, and metal halide bulbs, which provided plants with a 250 μmol m^{-2} s^{-1} of photosynthetically-active radiation (measured with a LI250A LICOR Biosciences light meter, Lincoln, NE, USA).

Experiment 1: pea nodulation under SRC and lime treatment

In a previous work with pea, SRC was found to have strong positive effects on nodulation; furthermore, nodulated plants were able to acquire sufficient nutrients for growth when provided only with the carbonatite and de-ionized water (Jones 2016). However, because control and SRC-treated plants were grown under different nutrient regimes and the substrate pH was not controlled for, it was unclear what caused the beneficial effect to nodulation. In order to clarify the effects of SRC on nodulation and nodulated plants, a three-factor experimental design was used with seeds randomly assigned to Cone-tainers™ with either: the base substrate (control), the base substrate with 1:10 SRC, or the base substrate with 1:15 calcitic lime. For each factor and experimental replicate, eight seeds were assigned, and two experimental replicates were completed.

Plants were harvested 28 days after planting (DAP) to allow sufficient time for nodulation to occur. At this time, plants were removed from the soil, the shoots and roots separated at the cotyledons, and the roots cleaned of all soil particles. Nodulation was quantified by counting the number of functional nodules (as estimated by their pinkish colouration, Bisseling et al. 1978) and by determining the total nodule dry weight (DW) per plant. Individual nodules were excised from roots, pooled together for each plant, and dried at 60°C for at least 72 h. The plant biomass (shoot and root DW) was then assessed after drying the samples as per the nodules. At harvest, the pH of homogenized substrate obtained from each Cone-tainer™ was measured on a 1:1 mixture of de-ionized water:substrate solution mixed for 5 seconds, then left to equilibrate for 10 minutes (Watson and Brown 1998). Finally, the concentration of macro-nutrients (N, P, K, Ca, Mg, S) and micro-nutrients (Fe, Al, Mn, B, Cu, Zn) in dried shoots was determined by independent analysis (Actlab Agriculture Laboratories, Ancaster, ON, Canada). Approximately 0.5 g of tissue dry weight was required for nutrient analysis, and in order to achieve this the shoot samples were pooled (2-3 plants/sample in each treatment group per trial). Shoot nutrient content was then calculated using the obtained ppm concentration values multiplied by the average shoot DWs of the pooled sample.

Several parameters were also calculated to gain further insight into how the amendments might be affecting plant growth and nodulation. As an indication of biomass investment responses to substrate nutrients (Gruber et al. 2013), the root to shoot (R:S) ratio was calculated by dividing the root DW by the shoot DW. A smaller R:S ratio shows that more biomass was invested in shoots than in roots, and vice versa with a larger R:S ratio. The plant and nodule DWs were also used to estimate the efficiency of the symbiotic association by calculating plant return on nodule construction cost (PRCC; host total DW/nodule DW; Oono and Denison 2010), specific nodulation (nodule number/root DW; Gulden and Vessey 1998) and specific nodule DW (nodule DW/host root DW; Gulden and Vessey 1998). A lower PRCC is associated with a more efficient symbiosis; plants acquire more biomass based on their investment in nodules. A higher specific nodulation and specific nodule DW also shows a more efficient symbiosis, with plants able to host more nodules and nodule biomass based on a given root system size.

Experiment 2: pea seedling growth under contrasting nutrient conditions

During a developmental study on pea involving SRC (Jones 2016), it was observed that the root biomass of SRC-treated plants increased over that of non-treated plants during early (~9 DAP) growth, but this response was transient. Again, it was unclear whether this response was unique to SRC, as different nutrient regimes were used for control and SRC-treated plants, and pH was not controlled for. To clarify this issue, an early growth experiment was conducted with seeds randomly assigned to one of four treatment groups: control substrates amended with lime (LC) or with SRC (SC), and those substrates prepared with additional nutrients (LH for lime Hoagland and SH for SRC Hoagland). The experiment was replicated three times (n = 8 seeds per treatment group per replicate). While the substrates already contained some nutrients, especially those which where amended with SRC, additional nutrients were provided using the modified Hoagland solution detailed above. Prior to planting, either water or the nutrient solution was mixed into the substrate to give a 20% (vol/vol) water content.

After two weeks of growth (14 DAP), plants were removed from soil, the roots were separated from the shoots, and the soil pH was determined as above. Because the responses seen previously with pea were exclusively root-based, the root system architecture was of special interest to us, and several root parameters were measured prior to drying and biomass determination. These included the length of the primary root, the number of lateral roots, and the length of the longest lateral root which were measured or quantified manually. Additionally, estimations of the overall root length and the total root surface area were obtained using the Arabidopsis WinRhizo™ software and the EPSON 10000XL 3.49 Flatbed scanner system (Regent Instruments Inc., Canada; software version 2012d). After drying, the R:S ratio was calculated as prior. Finally, the specific root length (SRL; m g^{-1} root DW) was calculated by dividing the root length by the root DW. The SRL gives insight into a plant's nutrient foraging strategy (Freschet and Roumet 2017; Wen et al. 2019), with low SRL values relating to thicker roots and more intensive nutrient acquisition in a given soil volume, and with high SRL values relating to thinner roots and a higher volume of soil exploited.

Experiment 3: interactions between microorganisms and carbonatite and effects on pea

To explore whether soil microorganisms respond to and/or alter plant responses to SRC, a two-factor, two-level experimental design was used. Seeds were randomly assigned to one of four treatment groups: control substrates amended with lime (LC) or SRC (SC), and the same substrates inoculated with 10 mL of an agricultural microorganism solution (LAG for lime with agricultural microbes and SAG for SRC with agricultural microbes) just after planting. For each level and factor, eight seeds were assigned per experimental replicate, and the experiment was replicated two times.

Plants were harvested after three weeks (21 DAP) during vegetative growth. The shoot and root biomass, the soil pH, the root length and surface area, and the shoot nutrient contents were measured, and the R:S ratio and the SRL calculated. Additionally, inoculated plants produced nodules, and so the nodule number and DW were determined. Finally, the abundance of culturable heterotrophic soil microorganisms was quantified via plating on a 1.5% nutrient agar medium (Difco

Nutrient Agar, pH 6.8) following Jones (2016). For this, a dilution series was made, beginning with a substrate extract consisting of 1 g of homogenized substrate with 9 mL of sterile de-ionized water. This extract was considered to be the initial 10^{-1} dilution, and further dilutions were prepared from it up to 10^{-4}. From each dilution, 100 µL were spread-plated under sterile conditions onto the agar plates; these were sealed with Parafilm® and incubated in the dark under growth room temperatures. The number of colonies was assessed daily and converted to colony-forming-units (CFU) per g soil by multiplying the number of colonies by the dilution factor, and then dividing by the volume plated. Counts were found to be optimal 48 h after plating using the 10^{-4} dilution; under the aforementioned conditions, countable CFUs were between 30 and 300 per plate.

Materials and Methods - Wheat

Growth conditions

Prior to planting, untreated seeds of spring wheat (*Triticum aestivum* L.) var. 'Norwell' were surface-sterilized and pre-germinated following aseptic procedures adapted from Sauer and Burroughs (1986) and Wu et al. (2007), respectively. Briefly, seeds were shaken for 30 sec in 95 % ethanol, and then rinsed twice with de-ionized water before being placed on Whatman #1 filter paper moistened with 6 mL of de-ionized water in 9 cm diameter Petri plates. Plates were then sealed with Parafilm®, and left under growth-room conditions (see pea methods) for 48 h. Germinated seeds were planted three per 3.78 L pot at a depth of 1 cm, and were thinned within 1 day of establishment to keep the largest emerged seedling per pot. Plants were grown in the greenhouse at the Cold Regions and Water Science Building at Wilfrid Laurier University. Average photoperiods, temperatures, and ambient humidity for the growing periods can be found in Table S4.2. Plants were watered individually from above every 2-4 days with de-ionized water, and were given chemical fertilizer (Miracle-Gro® commercial fertilizer at 2g/L, N:P:K of 22:14:6) every third watering after 14 days. All plants were inoculated with the agricultural microbial mixture immediately after planting; this was to help control

for variations caused by microorganisms from the external environment and to start each plant with the same microbial community.

Experiment 4: wheat growth and yield responses to carbonatite

As there is no information on how carbonatite use can affect the growth of wheat, here a full life-cycle study was conducted, including assessment of weekly growth and of final plant yields. A three-factor, two-level design was used where seeds were randomly assigned to one of three substrates: substrates amended with 1:10 silica sand:substrate (control), substrate amended with 1:15 calcitic lime:substrate, and substrate amended with 1:10 SRC:substrate. A total of fifteen plants were grown per factor, and for each factor, five plants were left to grow until senescence for yield determination. Two experimental replicates were completed, with trial one grown from Nov 22/2017 to Jan 17/2018, and trial two grown from Mar 18/2018 to May 13/2018 (Table S4.2a). The silica sand (grain size of ~ 1 mm; Bell and Mackenzie, Hamilton, ON, Canada) was used to control for changes in substrate structure which might be introduced by the other amendments, and to verify which effects were the result of changing substrate pH.

Each week following planting, plant development and growth was assessed by recording the Zadok's developmental stage (Zadoks et al. 1974). The length of the longest leaf (i.e., its blade and sheath) was also measured from the soil surface to the leaf tip; I called this parameter "shoot height" for simplicity. Plants were harvested 56 DAP which represented peak vegetative growth under these conditions. At this time, plants were removed from the soil, the shoots separated from the roots and the roots cleaned of all substrate particles. Two root characteristics were then determined to examine how SRC might be altering root architecture: the overall root length and the total root surface area. These were obtained using the Arabidopsis WinRhizo™ software and EPSON 10000XL 3.49 Flatbed scanner system (Regent Instruments Inc., Canada; software version 2012d). Following drying for at least 72 h at 60°C, the root and shoot DW were assessed. The concentration of macro-nutrients (N, P, K, Ca, Mg, S) and micro-nutrients (Fe, Al, Mn, B, Cu, Zn) in dried shoots was determined by

independent analysis (Actlab Agriculture Laboratories, Ancaster, ON, Canada). For yield measurements, five randomly-selected plants per treatment were left to grow until senescence; seeds were collected as the plants matured and the number and total dry weight of seeds per plant were determined. To gain insight into the effect of SRC and lime on soil microorganisms, the microbial activity of the substrate was determined through CO_2 evolution measured by alkali trap (Rowell 1995). Briefly, 50 g of fresh substrate collected during harvest (homogenized from each pot) were incubated in a Magenta™ jar with 10 mL of 1M NaOH separated in a small plastic vial. After 5-7 days incubation, to quantify the amount of CO_2 given off by the substrate and absorbed by the NaOH over the incubation period, the NaOH was titrated with 1M HCl and 1% phenolphthalein indicator (Sigma-Aldrich, Oakville, ON). Finally, the soil pH was measured on a 1:1 mixture of de-ionized water:soil solution mixed for 5 seconds, then left to equilibrate for 10 minutes (Watson and Brown 1998).

Several parameters were also calculated. As with pea, the R:S ratio and the SRL were used as indicators of biomass investment and nutrient foraging strategy, respectively. Using the weekly heights measured, growth rates for wheat (cm per unit time) were calculated through a linear regression (the "lm" function; R software suite version 3.5.2) to obtain the slope (growth rate) of each individual plant over time. The same linear regression was used between SRL and shoot nutrient contents to investigate the connection between altered root morphologies and nutrient uptake. It was hypothesized that wheat plants with less access to nutrients would have a higher SRL (i.e., more root length to exploit more soil volume) than those plants with greater nutrient access (less root length).

Experiment 5: early wheat growth and determination of nutrient-based effects from carbonatite

To test whether a nutrient-based mechanism was responsible for the effect of carbonatite on wheat, a two-factor, two-level design was used with seeds randomly assigned to either substrates amended with lime or SRC (LC and SC), or substrates amended and prepared with additional nutrients (LH for lime Hoagland and SH for SRC Hoagland). For each level and factor, ten plants

were used per experimental replicate. Two replicates were conducted; one from June 6/2018 to June 20/2018, and another from Oct 16/2018 to Oct 30/2018 (Table S4.2b). Additional nutrients were provided in the form of the modified Hoagland solution (Guinel and Sloetjes 2000) which was mixed in place of water into the substrate prior to planting (20% vol/vol with substrate). All plants were provided only with deionised water during growth to maintain 20% water content (roughly every 2-3 days). Each seed was planted individually in a 420 mL pot at a depth of 1 cm. Growth and development were measured daily as per the previous wheat experiment and plants were harvested at 14 DAP. The plant biomass, root characteristics (including root length, surface area, and SRL), R:S ratio, substrate pH, and daily growth rate were obtained as described above.

Statistical analyses

All statistical analyses were completed using the R software suite (version 3.5.2; http://www.r-project.org/). For pea the growing conditions were controlled between trials and considered identical. Therefore, trials were analyzed together if no effect of trial was observed. Differences in each parameter between treatments were tested for using a one-way ANOVA. When treatments were shown to be a significant source of variation (95 % confidence level), a Tukey Honest Significant Difference (HSD) post-hoc test was used to identify treatment-specific differences. Normality was tested using the Shapiro-Wilk test on residuals extracted from each ANOVA model. Since each treatment for pea had a single watering tray in each experiment, the plants were not fully independent from each other and were pseudoreplicates. This was accounted for by conducting multiple replicates of the same experiment. For wheat, trials were only considered separately due to differences in growing conditions. Because parameters for wheat were often not normally-distributed, the mean measurements between treatments were compared using a Kruskal-Wallis test followed by a post-hoc Dunn's test using the 'dunntest' function included in the FSA package (Ogle et al. 2019).

Results - Pea

Experiment 1: pea nodulation under SRC and lime treatment

At 28 DAP, the responses of pea to lime or carbonatite addition were subtle, and only the shoot dry weight (Figure 4.1), number of nodules (Table 4.1), and specific nodulation (Table 4.1) were altered. Typically, SRC had similar effects on nodulated pea to those of lime, although to a greater extent. The SRC-treated plants produced significantly more shoot biomass than control plants with shoots of lime-treated plants intermediate to both (Figure 4.1). The opposite was true for the number of nodules produced; control plants produced significantly more nodules than plants grown in SRC-amended substrates, and lime was again intermediate (Table 4.1). Finally, because SRC-treated plants had fewer nodules but similar root biomass to control plants, the specific nodulation in SRC-treated plants was also decreased compared to control plants (Table 4.1). No other differences were found between plants, although it is noteworthy that despite the differences in nodule numbers across plants in the different treatment groups, there were no significant differences in the nodule DW (Table 4.1). Additionally, both SRC and lime altered the substrate pH to a similar extent and increased it over that of the control substrates (Table 4.1). There were no differences in the macro-nutrient contents between treatments, with the exception of Ca which was highest in plants grown in SRC-amended substrates and lowest in control plants (Figure 4.2). However, differences in the shoot micro-nutrient contents were observed. For instance, the Mn content of SRC-treated plants was significantly higher than that of control plants, with lime-treated plants intermediate in their values (Figure 4.2). The opposite pattern was found with Zn contents, where control plants acquired the most Zn and SRC-treated plants the least, with lime-treated plants again intermediate (Figure 4.2). For B and Cu, different patterns were observed. For B, control plants had significantly higher shoot contents than both SRC-treated and lime-treated plants (Figure 4.2), and for Cu, SRC-treated plants had higher shoot contents than lime-treated plants, with control plants intermediate (Figure 4.2).

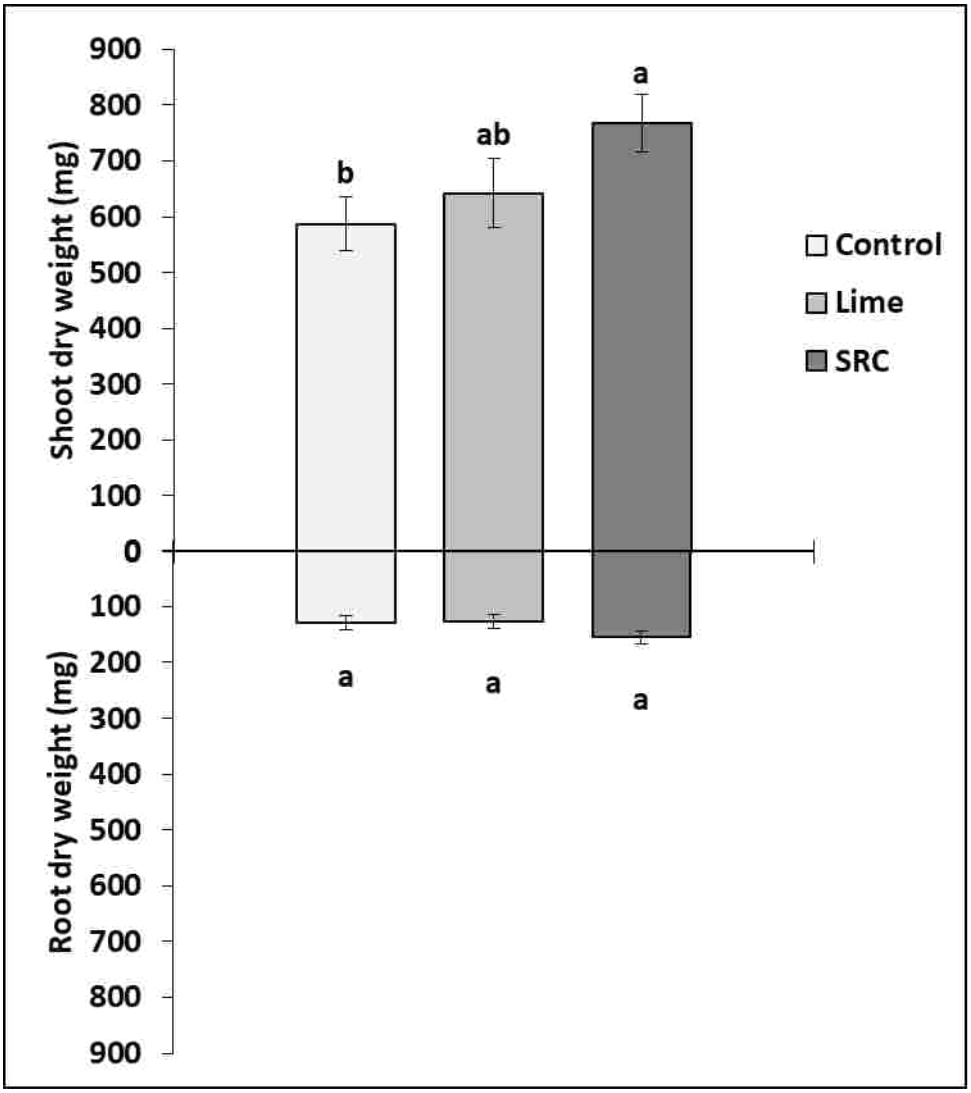


Figure 4.1: Shoot and root biomass of 28 day-old inoculated peas that were grown in substrates with either no amendment (control), 1:15 calcitic lime:substrate, or 1:10 SRC:substrate. Values are given as means ± standard error (n = 16 plants per treatment). Statistically-significant results between treatments are indicated with differing superscripted letters (one-way ANOVA + Tukey HSD post hoc test at 95% confidence level). Root dry weight does not include nodules.

Table 4.1: Root:shoot ratios, nodulation parameters, and substrate pH for 28 day-old nodulated pea plants grown in substrates with either no amendment (control), 1:15 calcitic lime:substrate, or 1:10 SRC:substrate. Values are given as means ± standard error (n = 16 plants per treatment). Statistically-significant results between treatments are indicated with differing superscripted letters (one-way ANOVA + Tukey HSD post hoc test at 95% confidence level). R:S = root:shoot, NDW = nodule dry weight, PRCC = plant return on construction cost, Nod. = nodulation.

	Control	Lime	SRC
R:S ratio	0.22 ± 0.01 **a**	0.20 ± 0.01 **a**	0.20 ± 0.01 **a**
Number of nodules	186.4 ± 14.8 **a**	141.3 ± 13.3 **ab**	134.2 ± 18.5 **b**
NDW (mg)	51.73 ± 4.16 **a**	55.92 ± 5.50 **a**	67.26 ± 4.76 **a**
PRCC	14.21 ± 0.67 **a**	13.98 ± 0.73 **a**	13.98 ± 0.52 **a**
Specific NDW	407.14 ± 21.60 **a**	453.03 ± 18.82 **a**	441.56 ± 27.98 **a**
Specific Nod.	1510.29 ± 107.01 **a**	1260.94 ± 147.13 **ab**	899.42 ± 116.84 **b**
pH	5.63 ± 0.02 **b**	7.11 ± 0.03 **a**	7.11 ± 0.02 **a**

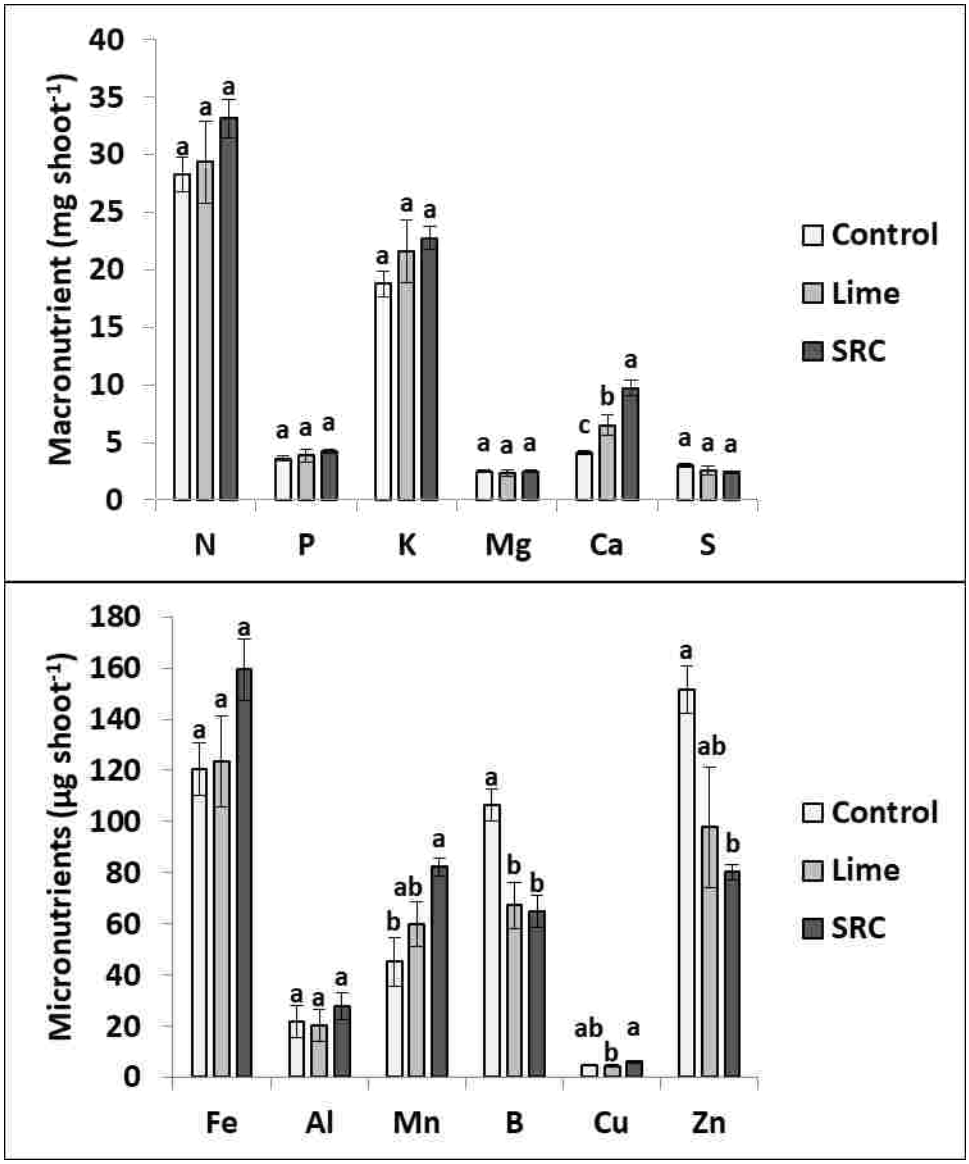


Figure 4.2: Mean nutrient content (± standard error) of 28 day-old pea shoots. Nodulated plants were given either no amendment (control), calcitic lime (1:15 lime:substrate), or SRC (1:10 SRC:substrate). Within each treatment, the dried shoots were pooled before analysis to give 6 samples per treatment group across the two trials. Significant differences between treatment groups for each element are indicated by different letters (one-way ANOVA and Tukey HSD tests at 95% confidence). Nutrient content was calculated by multiplying the average shoot dry weights of the pooled samples by the ppm concentrations for each element.

Experiment 2: pea seedling growth under contrasting nutrient conditions

Altering the substrate nutrient content and amendments caused minimal changes to 14 day-old-peas plants, and only in the R:S ratio and the substrate pH were differences observed (Table 4.2). Although there were no differences in the shoot or root biomass across treatments (Figure 4.3), SH plants had significantly lower R:S ratios than LC plants with plants of the other two treatments intermediate (Table 4.2). Despite this, no differences in any root architecture parameters measured were noted across treatment groups (Table 4.2). Finally, the additional nutrients had no effect on substrate pH although overall the lime-amended substrates had slightly higher pHs than SRC-treated substrates (Table 4.2).

Table 4.2: The root to shoot ratio, root architecture parameters and substrate pH of 14 day-old pea grown in control substrates amended with lime or SRC without any additional nutrients (LC and SC, respectively), or in those substrates prepared with additional nutrients (LH for lime Hoagland and SH for SRC Hoagland). Values presented as means ± standard error (n = 14-24 per treatment across three trials). Significant differences between treatment groups for each element are indicated by different letters (One-way ANOVA at 95 % confidence level). R:S = root:shoot, SRL = specific root length, LR = lateral root, PR = primary root, SA = surface area.

	LC	LH	SC	SH
R:S ratio	0.80 ± 0.02 **a**	0.75 ± 0.04 **ab**	0.73 ± 0.03 **ab**	0.68 ± 0.03 **b**
SRL (m g^{-1} RDW)	43.13 ± 2.03 **a**	43.82 ± 2.07 **a**	44.73 ± 1.67 **a**	49.79 ± 2.50 **a**
Number of LRs	33.14 ± 3.67 **a**	34.42 ± 3.47 **a**	34.96 ± 2.56 **a**	41.36 ± 2.56 **a**
Longest LR (cm)	11.74 ± 0.51 **a**	10.68 ± 0.57 **a**	11.38 ± 0.55 **a**	11.20 ± 0.73 **a**
PR length (cm)	14.81 ± 2.22 **a**	18.53 ± 2.01 **a**	18.42 ± 1.38 **a**	20.88 ± 0.58 **a**
Total root length (cm)	193.61 ± 17.28 **a**	196.49 ± 17.94 **a**	205.42 ± 14.30 **a**	222.65 ± 21.95 **a**
Total SA (cm^2)	32.51 ± 4.17 **a**	31.28 ± 4.82 **a**	35.92 ± 4.08 **a**	35.35 ± 5.64 **a**
pH	7.63 ± 0.03 **a**	7.52 ± 0.03 **a**	7.38 ± 0.03 **b**	7.45 ± 0.02 **b**

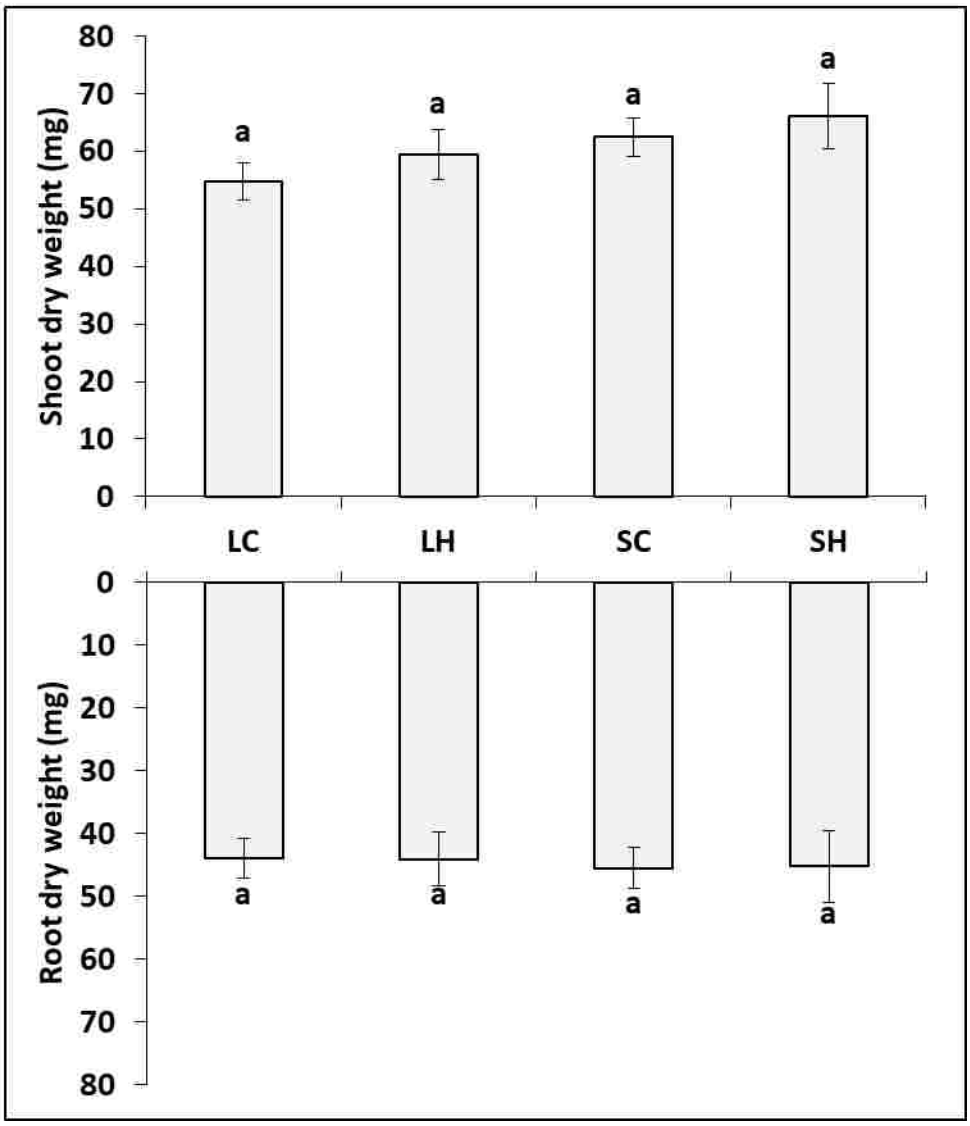


Figure 4.3: Shoot and root biomass of 14 day-old pea grown in control substrates amended with lime or SRC without any additional nutrients (LC and SC, respectively), or in those substrates prepared with additional nutrients (LH for lime Hoagland and SH for SRC Hoagland). Values presented as means ± standard error (n = 14-24 per treatment across three trials). Significant differences between treatment groups for each element are indicated by different letters (One-way ANOVA at 95 % confidence level).

Experiment 3: interactions between microorganisms and carbonatite and effects on pea

Results obtained with 21 DAP pea demonstrate a complex root-based response to carbonatite amendment and agricultural microbial inoculation. No differences in shoot biomass were seen across treatment groups (Figure 4.4). Inoculation with microbes did not change root biomass within treatments, but SC root systems had significantly more biomass than LAG root systems (Figure 4.4). However, no changes were seen in terms of R:S ratios among treatments (Table 4.3). Largely the root architecture parameters followed the changes in root biomass, although small differences were noted. One such difference was in the total root system length where the SAG root systems were significantly longer than LAG root systems, with the SC and LC root systems of intermediate length (Table 4.3). For total root surface area, the SRC-treated plants, inoculated or not, had significantly more surface area than inoculated lime-treated plants, and LC-plants were intermediate (Table 4.3). The pH between all substrates was largely identical, although it was significantly increased in LAG substrates compared to the other treatments (Table 4.3). Plants inoculated with agricultural microorganisms produced nodules, although there were no significant differences between amendments in terms of nodule numbers (~22 per root system) or nodule dry weight (~7.23 mg total per plant). No differences were observed in either macro- or micro-nutrient contents across treatments (Figure 4.5).

The SRL and the number of CFU highlight the complexity of the three-way interaction between pea, amendment, and soil microorganisms. The SRL of 21 day-old pea plants did not change with lime or inoculation (~ 86 m g^{-1} root DW), but was significantly higher in inoculated (~ 87 m g^{-1} root DW) than in non-inoculated (~74 m g^{-1} root DW) plants grown in SRC-amended substrates (Figure 4.6a). There were significantly lower numbers of culturable heterotrophic CFUS in lime-amended substrates which were inoculated with agricultural microorganisms than in those lime-amended substrates which were not inoculated (Figure 4.6b). In SRC-amended substrates, inoculation had no effect on the number of heterotrophic CFUs, which were intermediate to those of both lime-amended substrates (Figure 4.6b).

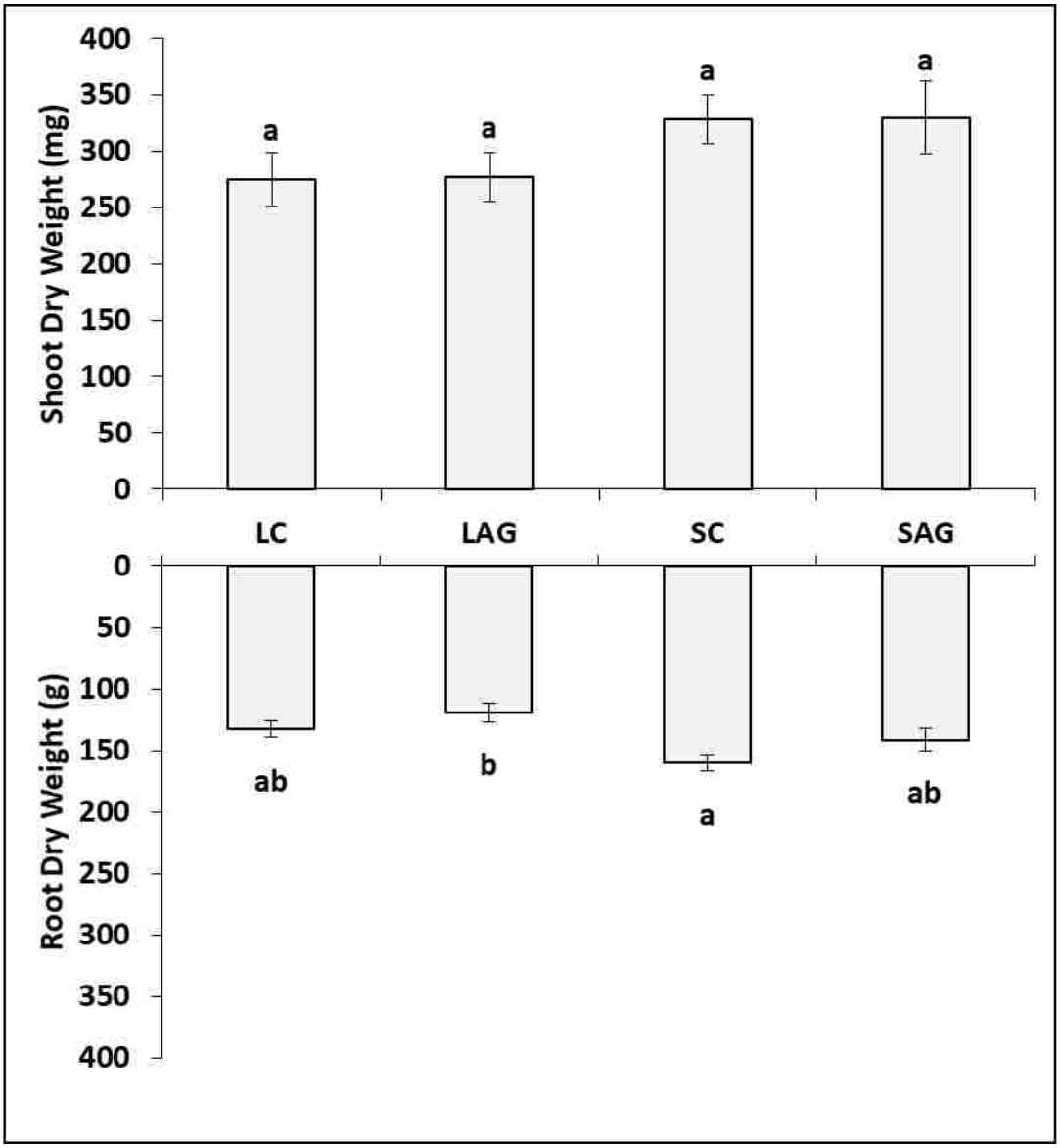


Figure 4.4: Shoot and root biomass parameters of 21 day-old pea grown either in control substrates amended with lime (LC) or SRC (SC), or the same substrates inoculated with an agricultural microorganism solution (LAG for lime with agricultural microbes and SAG for SRC with agricultural microbes). Values presented as means ± standard error (n = 14-15 per treatment). Significant differences between treatment groups for each element are indicated by different letters (One-way ANOVA at 95 % confidence level).

Table 4.3: R:S ratio, root architecture and substrate pH of 21 day-old pea grown either in control substrates amended with lime (LC) or SRC (SC), or the same substrates inoculated with an agricultural microorganism solution (LAG for lime with agricultural microbes and SAG for SRC with agricultural microbes). Values presented as means ± standard error (n = 14-15 per treatment). Significant differences between treatment groups for each element are indicated by different letters (One-way ANOVA at 95 % confidence level). R:S = root:shoot, RL = root length, RSA = root surface area.

	LC	LAG	SC	SAG
R:S ratio	0. 51 ± 0.03 **a**	0.44 ± 0.02 **a**	0.50 ± 0.02 **a**	0.46 ± 0.03 **a**
Total RL (cm)	1163.26 ± 70.00 **ab**	1006.09 ± 46.67 **b**	1190.78 ± 46.04 **ab**	1207.52 ± 60.66 **a**
Total RSA (cm^2)	228.58 ± 12.99 **ab**	202.28 ± 10.82 **b**	251.43 ± 11.11 **a**	247.88 ± 15.38 **a**
pH	7.07 ± 0.05 **b**	7.35 ± 0.02 **a**	7.12 ± 0.02 **b**	7.20 ± 2.55 **b**

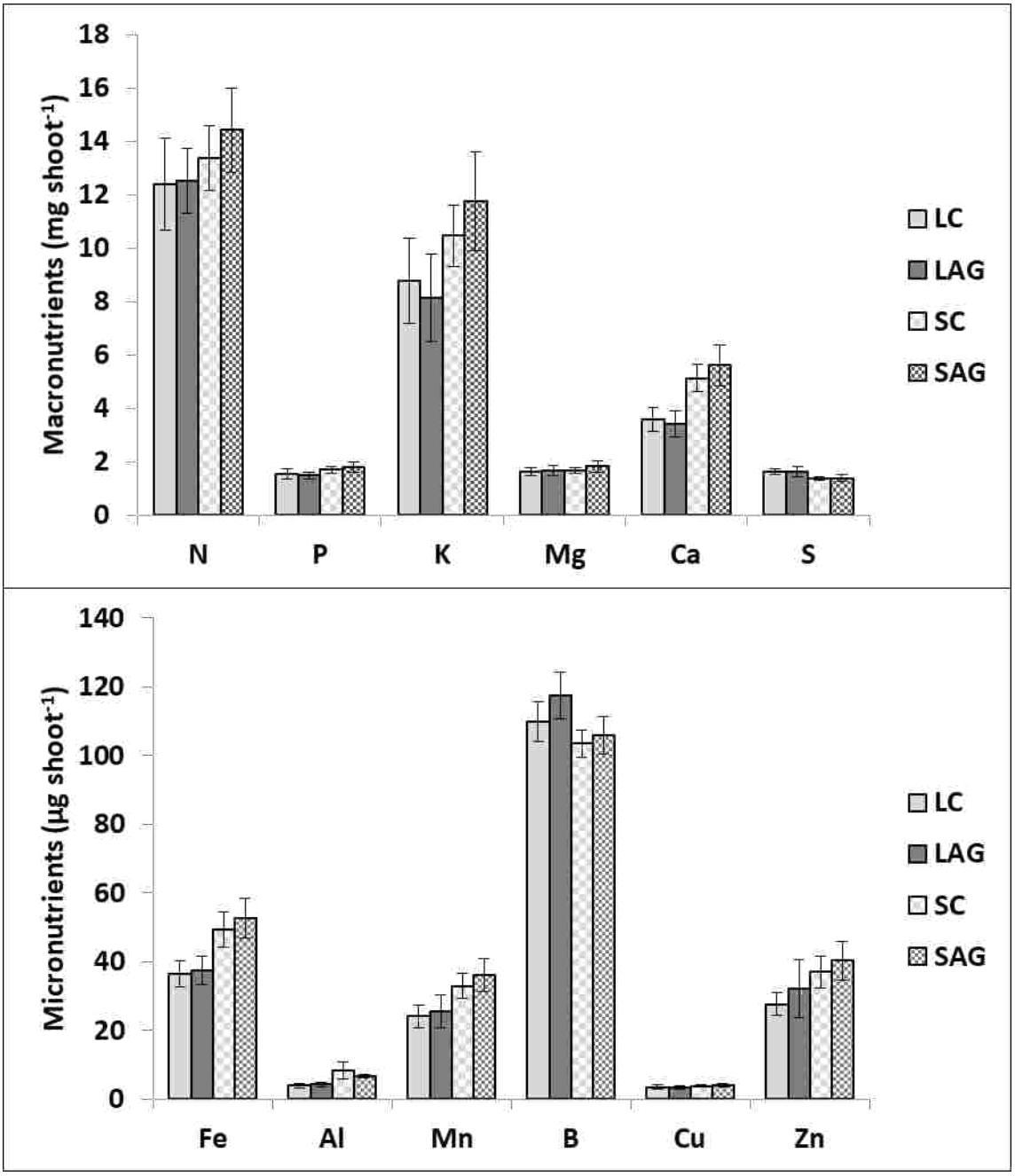


Figure 4.5: Average macro and micronutrient content of 21 day-old pooled pea shoots. Plants were grown in substrates with either lime (L) or SRC (S) and inoculated or not with an agricultural microbial solution (AG or C, respectively). Within each treatment, the dried shoots were pooled before analysis to give 6 samples per treatment group across the two trials. No significant differences between treatment groups were detected (One-way ANOVA and Tukey HSD tests at 95% confidence). Nutrient content was calculated by multiplying the average shoot dry weights of the pooled samples by the ppm concentrations for each element.

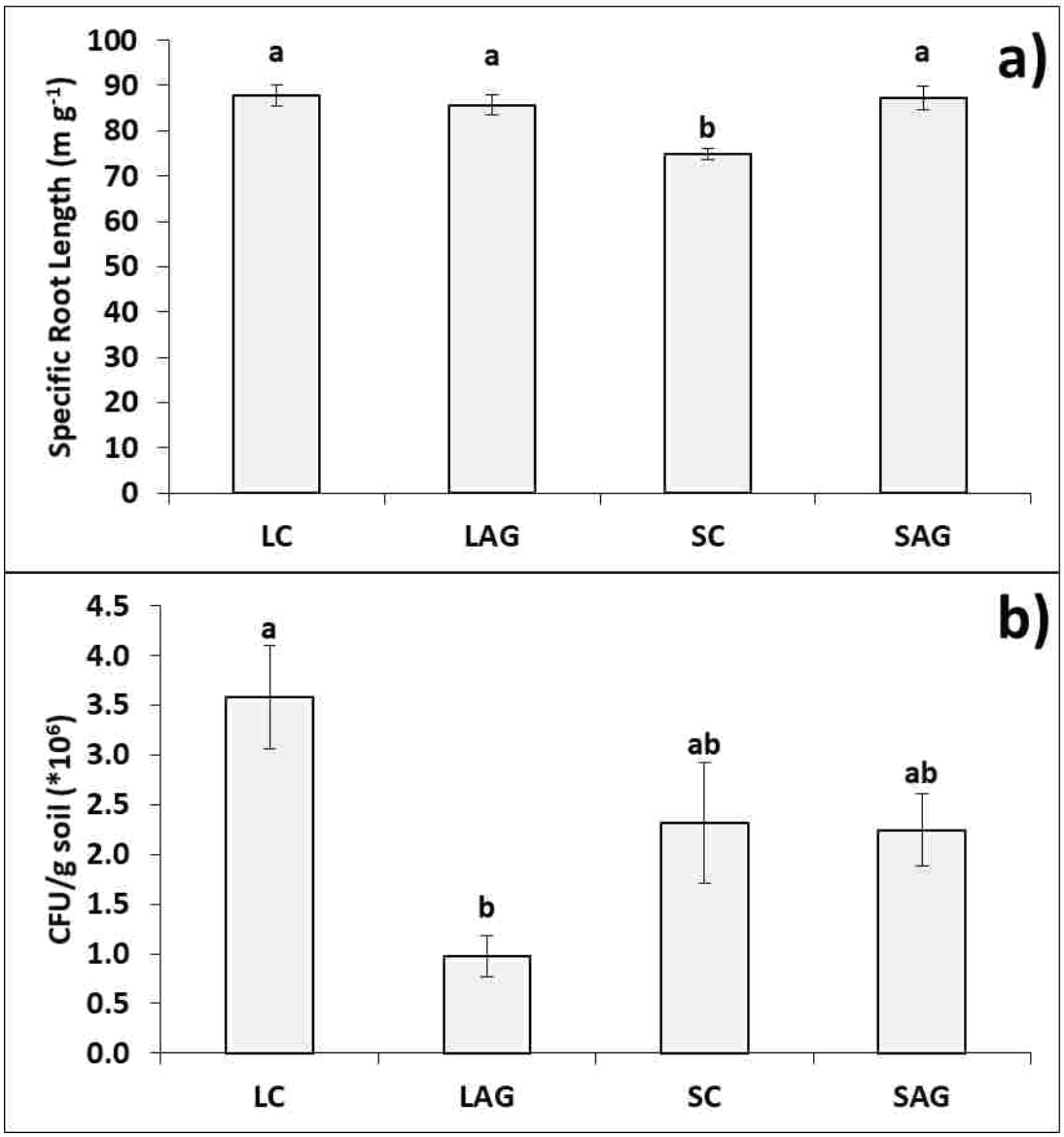


Figure 4.6: Specific root length (**a**) and number of heterotrophic CFU (**b**) from 21 day-old pea grown either in control substrates amended with lime (LC) or SRC (SC), or the same substrates inoculated with an agricultural microorganism solution (LAG for lime with agricultural microbes and SAG for SRC with agricultural microbes). Values presented as means ± standard error (n = 14-15 per treatment). Significant differences between treatment groups for each element are indicated by different letters (One-way ANOVA at 95 % confidence level). The specific root length was calculated for each plant by dividing the root length by the root biomass.

Results - wheat:

Experiment 4: wheat growth and yield responses to carbonatite

Each trial was analyzed separately due to environmental differences (e.g., in photoperiod; Table S4.2). Largely the same statistical trends were observed across both trials, although for trial one there were much greater differences between plants grown with SRC and those grown with lime and silica sand than for trial two. However, because trial two took place under growing conditions that are more optimal for wheat (e.g., longer photoperiods; see FAO Ecocrop profile for wheat), I chose to present only this trial as representative. Differences between trials will be noted where applicable.

Wheat responded rapidly and positively to the presence of SRC, and differences in plant growth were first observed after two weeks of growth. At this time, plants grown in SRC-amended substrates had noticeably increased shoot heights over those grown in lime-amended or control substrates for which the shoot heights were nearly identical (Figure 4.7). This pattern continued across the eight-week growing period, although the difference in height between the SRC-treated plants and those of the other treatments was maximal at three weeks of growth and maintained thereafter (Figure 4.7). The average calculated growth rate for plants in SRC-amended substrates was 1 cm week^{-1} higher (9.2 ± 0.1 cm week^{-1}) than that of control plants (8.3 ± 0.02 cm week^{-1}) or plants grown in lime-amended substrates (8.2 ± 0.3 cm week^{-1}). This was also observed, albeit to a greater extent, in the first trial where the plants grown in SRC-amended substrates grew 3.5 cm more per week (8.9 ± 0.2 cm week^{-1}) than those of control plants (5.5 ± 0.2 cm week^{-1}) or plants in lime-amended substrates (5.1 ± 0.2 cm week^{-1}). The increase in shoot heights was reflected in the biomass of plants at 56 DAP; at that time the shoot biomass of SRC-treated plants was nearly 66% more than those of lime-treated or control plants, and the root biomass 50% greater (Figure 4.8a). Roots of SRC-treated plants were visibly more robust than those of the other treatment groups, with more numerous lateral roots, a higher density of roots per unit volume, and more nodal roots emerging from the shoot base (Figure 4.8b). The differences in root architecture between plants grown with or without SRC were evident in the overall root lengths and total surface areas, which were significantly higher in plants grown with

SRC than those grown without (Table 4.4). Of particular note was the SRL, indicative of root thickness and nutrient-foraging strategy, which was significantly lower in SRC-treated plants than in plants of the other treatments (Table 4.4). This signifies that plants grown with SRC had thicker roots than those plants that were grown without SRC. After eight weeks of growth, lime- and SRC-treated substrates maintained a higher pH than that of the silica sand controls, though lime was more effective at raising the pH (Table 4.4). The microorganism activity between lime- and SRC-amended substrates was also different; substrates amended with lime exhibited a larger release of CO_2 than both SRC-amended and control substrates in trial two (Table 4.4). In trial one all substrates gave off equal amounts of CO_2 (~ 1.25 ± 0.01 ng CO_2 g^{-1} soil DW s^{-1}). Yield was unaltered by amendment with SRC or lime for the second trial; plants produced an average of 74 seeds each, with a combined average seed dry weight of 2.57 g per plant. This was in contrast to the first trial in which SRC-treated plants had a higher yield than lime-treated or control plants (Table S4.3).

The boosted growth of SRC-treated plants was also reflected in the shoot nutrient contents, where several macro-nutrients were present in higher quantities than in the other treatment groups (Figure 4.9), with the exceptions of Mg, Ca, and S which were found in similar amounts in lime-treated plants. The micro-nutrient contents were more variable, and B levels were notably lower in SRC-treated plants than in plants in other treatments (Figure 4.9). Levels of Zn were decreased in those plants grown in lime-amended substrates compared to those of the other treatments. The only micronutrient of which plants grown with SRC has twice the content of those plants grown in the other treatment groups was Mn (Figure 4.9).

A relationship between the SRL and each of the shoot nutrient measured was noticeable. This relationship was positive or negative depending on the element and trial, but was consistently negative and the strongest with plants grown in SRC-amended substrates (Figure 4.10). I chose here to focus on the three major components of the chemical fertilizer (N, P, K) and two elements easily attributable to SRC (Ca, Mn) although most of the macro- and micro-nutrient contents showed some relationship with SRL (Figure S4.1). Furthermore, the connection between the SRL and the nutrient content in plants with SRC was the greatest (as expressed by the R^2 values) in trial one (Figure 4.10);

this is because the differences in biomass between plants grown with SRC and those grown with lime or sand were the highest in trial one, and because the SRL and nutrient contents were derived from biomass parameters. Given that the trial one data were meaningful and different from those of trial two, I chose to present both trials for this relationship.

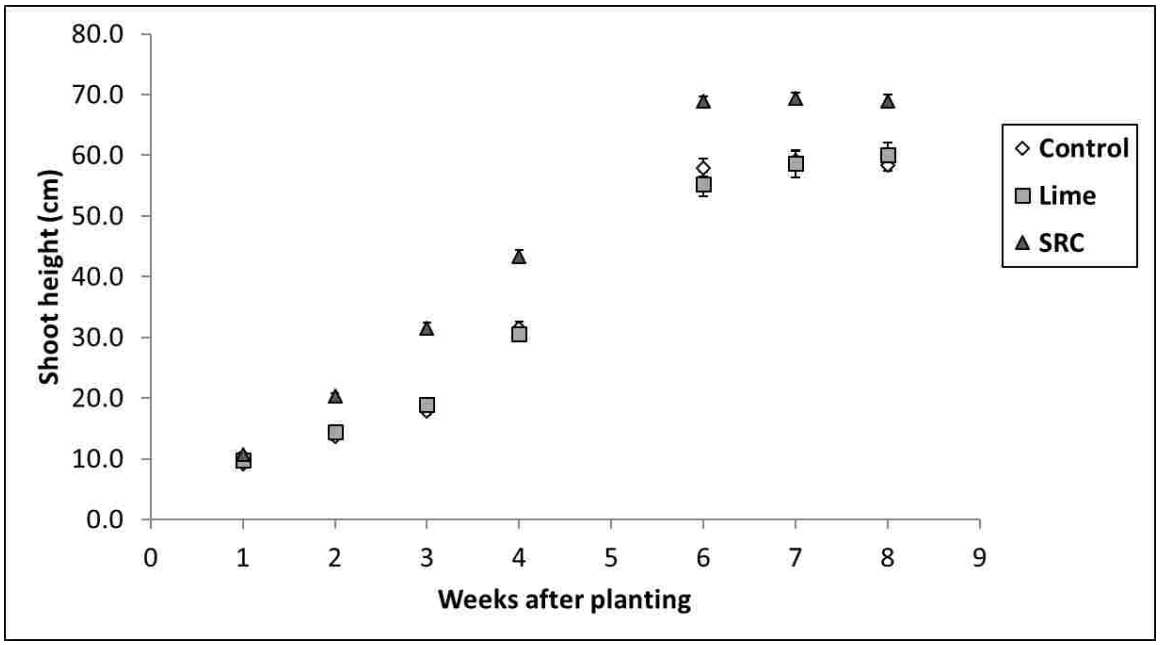


Figure 4.7: Average weekly shoot height of wheat (± standard error) over the eight-week growing period for trial two. Plants (n = 14-15 per treatment) were grown in substrates with either 1:10 silica sand:substrate (control), 1:15 calcitic lime:substrate (lime), or 1:10 SRC:substrate (SRC). Data were not obtained for week 5 because a local ice storm prevented measurements.

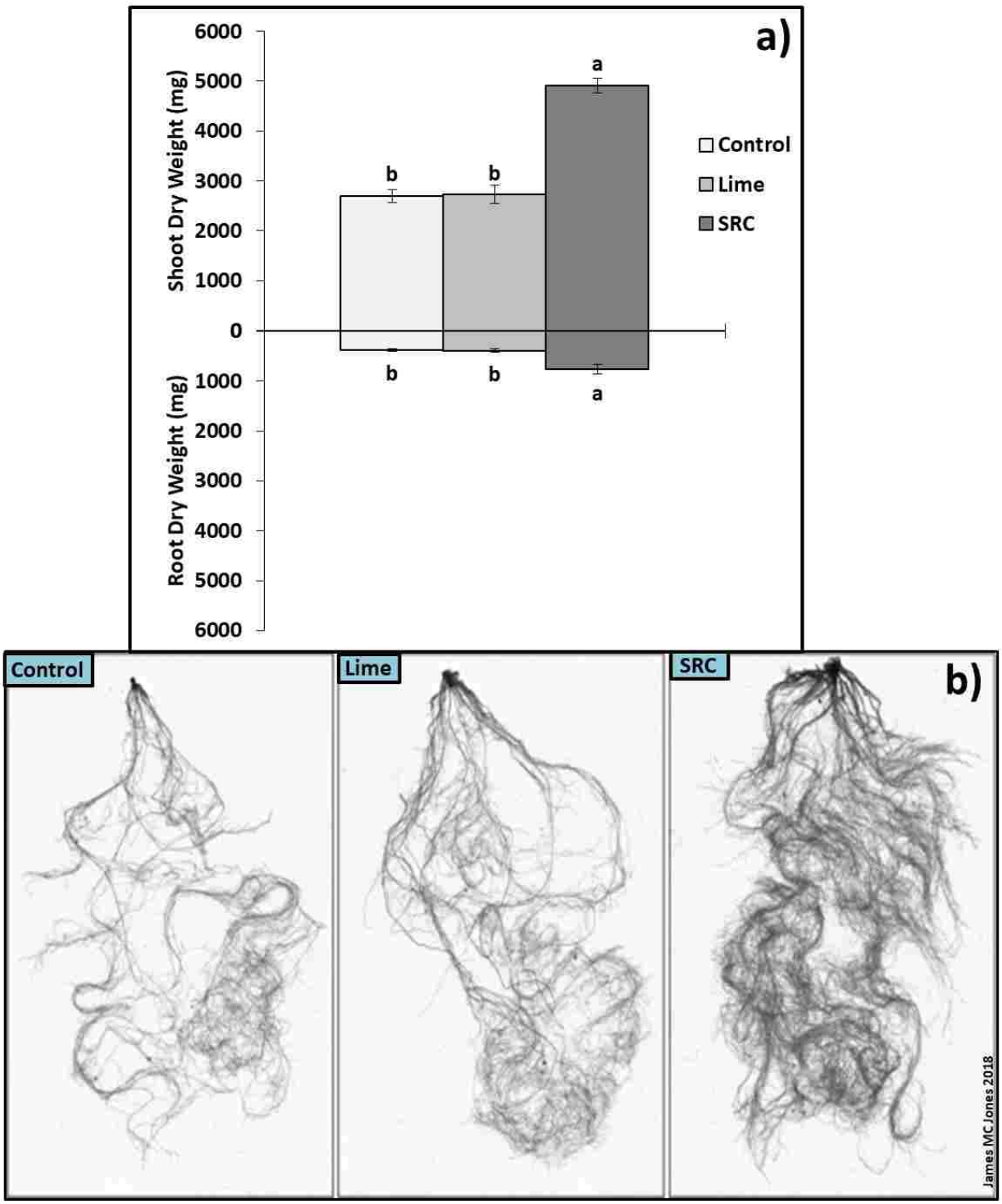


Figure 4.8: Shoot and root biomass (a) and representative root system scans (b) of 56 day-old wheat plants (n = 10 per treatment) from trial two. Plants were grown in substrates with either 1:10 silica sand:substrate (control), 1:15 calcitic lime:substrate (lime), or 1:10 SRC:substrate (SRC). Significant differences in biomass are indicated by different letters (Kruskal-Wallis + post-hoc Dunn's test at 95% confidence level).

Table 4.4: Root architecture parameters, and substrate parameters of 56 day-old wheat from trial two. Plants were grown in substrates amended with either 1:10 silica sand:substrate (control), 1:15 calcitic lime:substrate (lime), or 1:10 SRC:substrate (SRC). Respiration was used to indicate the microbial activity of the substrate. Significant differences are indicated by different letters (Kruskal-Wallis + post-hoc Dunn's test at 95% confidence level). RL = root length, RSA = root surface area, SRL = specific root length, DW = dry weight.

	Control	**Lime**	**SRC**
Total RL (cm)	3683.02 ± 199.91 **b**	4085.41 ± 204.63 **b**	4906.35 ± 168.80 **a**
Total RSA (cm^2)	133.75 ± 9.88 **b**	128.21 ± 8.95 **b**	187.41 ± 7.13 **a**
SRL (m g^{-1} root DW)	100.37 ± 4.80 **a**	110.35 ± 5.87 **a**	61.37 ± 5.63 **b**
Respiration (ng CO_2 g^{-1} DW s^{-1})	0.03 ± 0.01 **b**	0.10 ± 0.01 **a**	0.03 ± 0.01 **b**
pH	5.89 ± 0.03 **c**	7.66 ± 0.05 **a**	7.27 ± 0.03 **b**

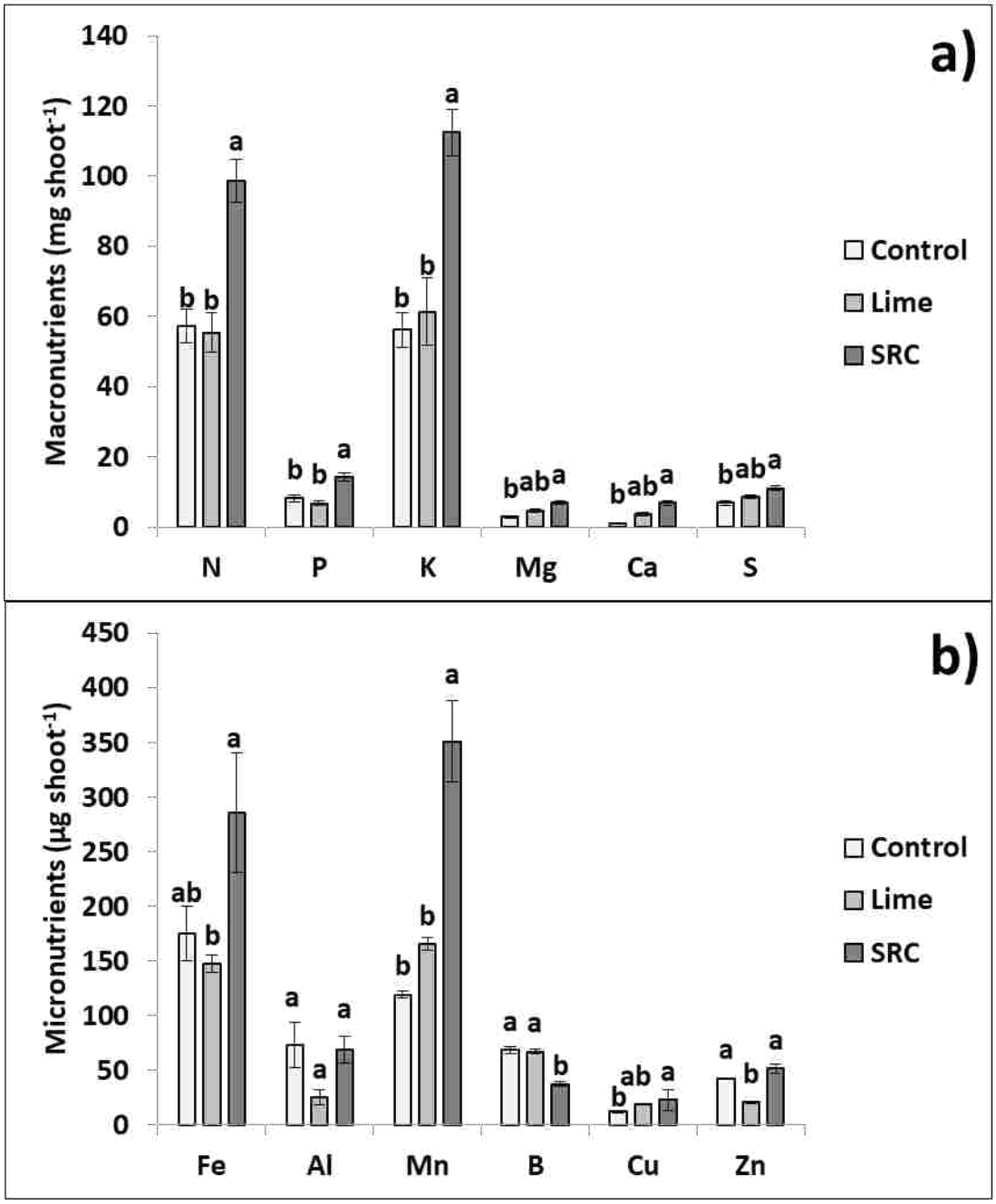
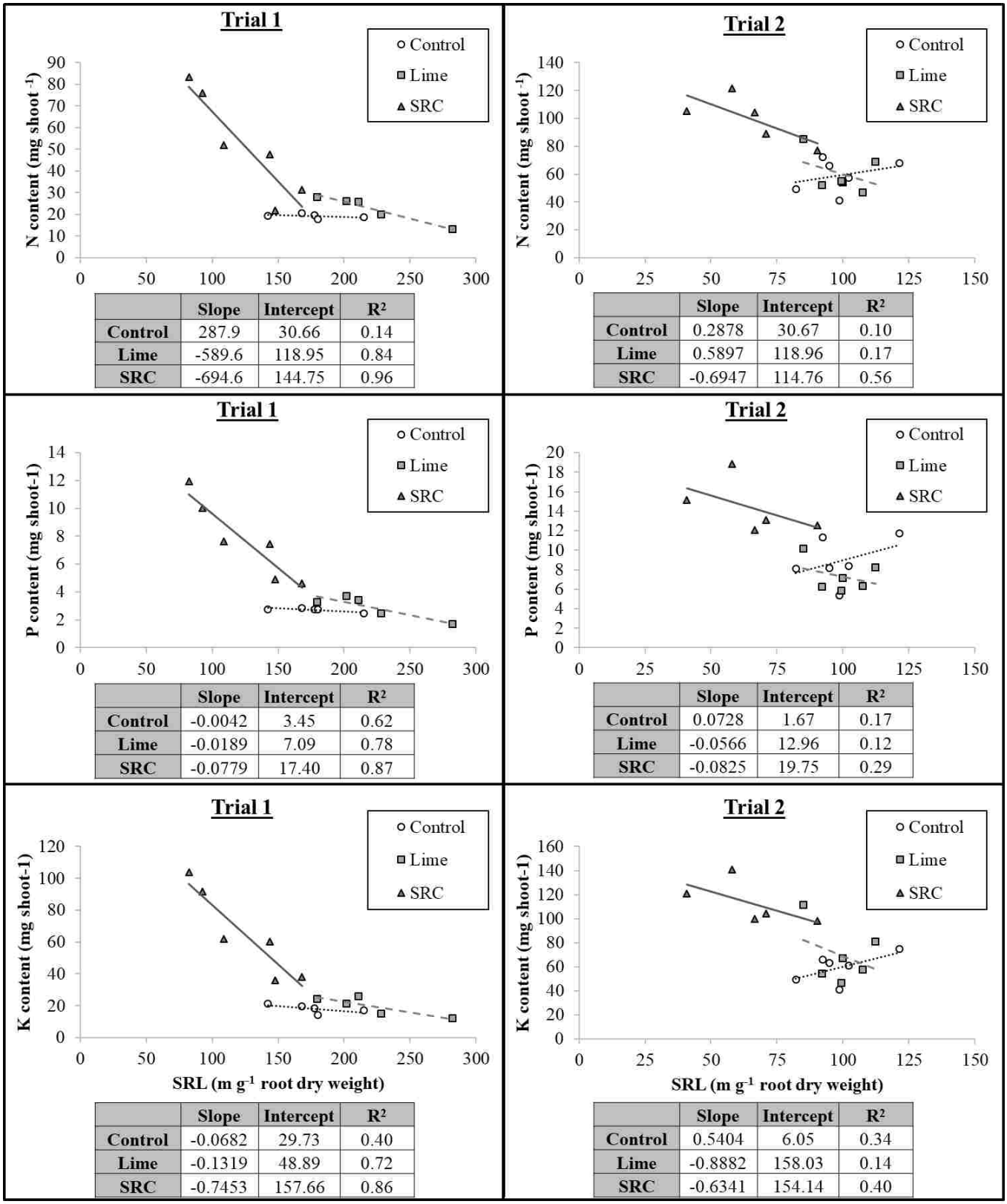


Figure 4.9: Average shoot macro-(a) and micro-nutrient (b) contents of 56 day-old wheat. Plants were grown in substrates amended with either lime, SRC, or silica sand (control). Significant differences between treatment groups for each element are indicated by different letters (Kruskal-Wallis and Dunn's tests at 95% confidence). Nutrient content was calculated by multiplying the shoot dry weights by the ppm concentrations for each element. N = 5-6 samples per treatment per trial.



	Slope	Intercept	R^2
Control	287.9	30.66	0.14
Lime	-589.6	118.95	0.84
SRC	-694.6	144.75	0.96

	Slope	Intercept	R^2
Control	0.2878	30.67	0.10
Lime	0.5897	118.96	0.17
SRC	-0.6947	114.76	0.56

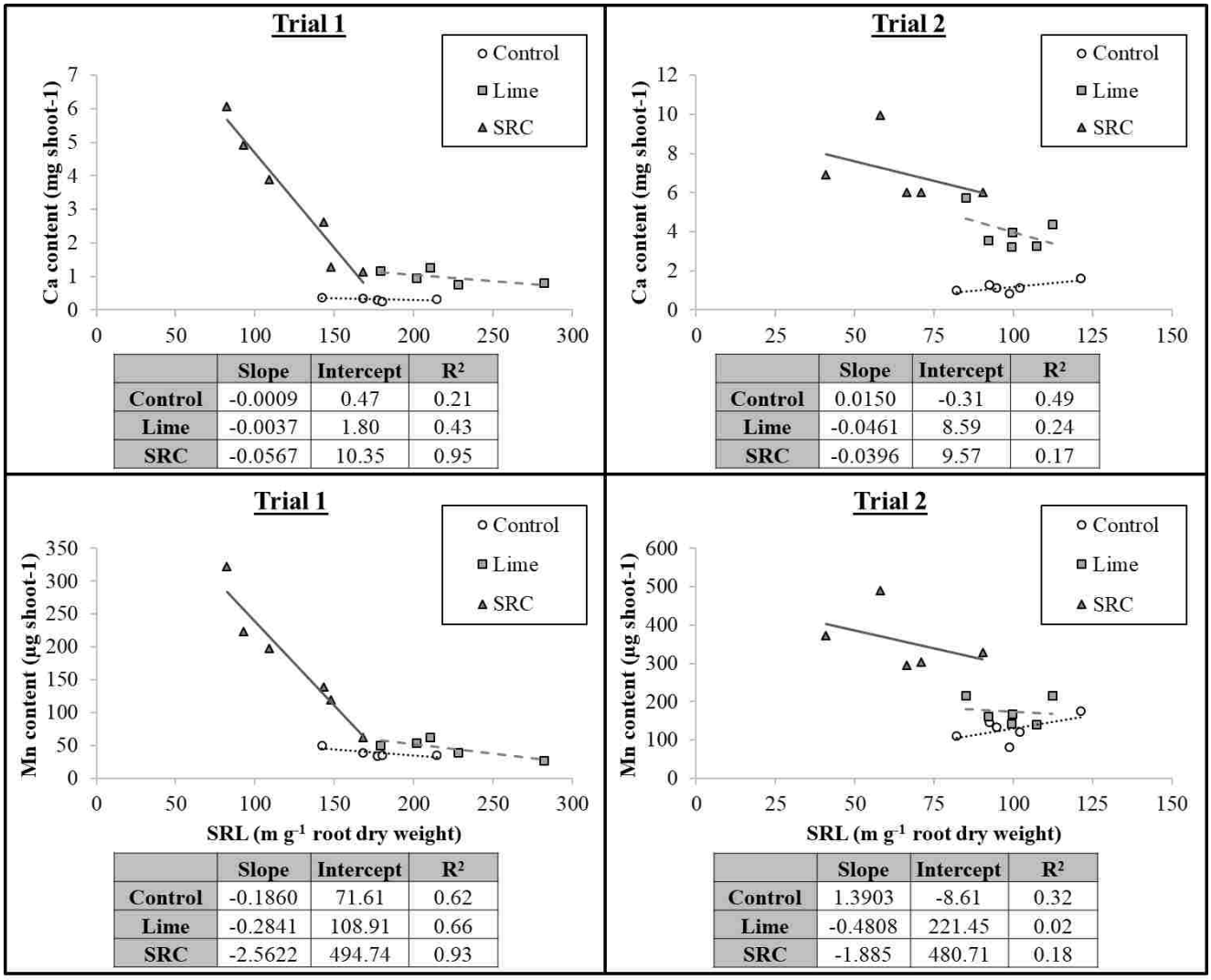
	Slope	Intercept	R^2
Control	-0.0042	3.45	0.62
Lime	-0.0189	7.09	0.78
SRC	-0.0779	17.40	0.87

	Slope	Intercept	R^2
Control	0.0728	1.67	0.17
Lime	-0.0566	12.96	0.12
SRC	-0.0825	19.75	0.29

	Slope	Intercept	R^2
Control	-0.0682	29.73	0.40
Lime	-0.1319	48.89	0.72
SRC	-0.7453	157.66	0.86

	Slope	Intercept	R^2
Control	0.5404	6.05	0.34
Lime	-0.8882	158.03	0.14
SRC	-0.6341	154.14	0.40

Figure 4.10



	Slope	Intercept	R^2
Control	-0.0009	0.47	0.21
Lime	-0.0037	1.80	0.43
SRC	-0.0567	10.35	0.95

	Slope	Intercept	R^2
Control	0.0150	-0.31	0.49
Lime	-0.0461	8.59	0.24
SRC	-0.0396	9.57	0.17

	Slope	Intercept	R^2
Control	-0.1860	71.61	0.62
Lime	-0.2841	108.91	0.66
SRC	-2.5622	494.74	0.93

	Slope	Intercept	R^2
Control	1.3903	-8.61	0.32
Lime	-0.4808	221.45	0.02
SRC	-1.885	480.71	0.18

Figure 4.10 continued: Linear regression analysis of specific root length (SRL) and shoot nutrient content in 56 day-old wheat plants (n = 5 per treatment group per trial) grown in either 1:10 silica sand (control), 1:15 calcitic lime:substrate (lime), or 1:10 SRC:substrate (SRC). Linear regression values were derived using the 'lm' function (R software suite version 3.5.2). Only the nutrients in the N:P:K fertilizer or those that could be easily attributable to SRC (Ca, Mn) are presented.

Experiment 5: early wheat growth and determination of nutrient-based effects from carbonatite

As with experiment 4, the two trials performed with wheat were analysed separately, and only trial 1 will be presented as representative with differences between trials noted where applicable. Both nutrient addition and amendment influenced the growth of two-week-old wheat. At this stage, differences in biomass among treatments were not apparent (Table 4.5). However, plants grown in substrates which had both SRC and additional nutrients added (SH) grew by nearly 1cm more per day than plants grown in substrate where just lime had been added (Table 4.5). The growth rates of plants in substrates amended with lime and nutrients and of plants grown in substrates with SRC were intermediate to those of plants in the aforementioned treatment groups. While no differences in root length were observed, both root surface area and SRL responded to the amendments and the addition of nutrients. For both parameters, the trend was that plants with access to the most amount of nutrients (i.e., SH plants) had higher surface area and SRL than plants with access to the least amount of nutrients (i.e., LC plants) (Table 4.5). Across all treatments, substrate pH was comparable, although for SC substrates it was lower than that of the other treatment groups (Table 4.5). Many of these trends were consistent in trial 2, although there the LC plants performed the weakest across most parameters (Table S4.4).

Table 4.5: Biomass, growth, and root architecture parameters of 14 day-old wheat (n = 8-10 plants per treatment) from trial one grown in substrates amended with lime or SRC (LC and SC), or substrates amended and prepared with additional nutrients (LH for lime Hoagland and SH for SRC Hoagland). Significant differences are indicated by different letters (Kruskal-Wallis + post-hoc Dunn's test at 95% confidence level). DW = dry weight, GR = growth rate, RL = root length, RSA = root surface area, SRL = specific root length.

	LC	SC	LH	SH
Shoot DW (mg)	40.82 ± 4.44 **a**	44.46 ± 5.76 **a**	43.21 ± 3.94 **a**	54.44 ± 4.35 **a**
Root DW (mg)	30.42 ± 2.79 **a**	36.00 ± 5.02 **a**	33.42 ± 2.94 **a**	37.73 ± 2.36 **a**
GR (cm day^{-1})	1.44 ± 0.11 **b**	1.68 ± 0.11 **ab**	1.86 ± 0.09 **ab**	2.18 ± 0.09 **a**
Total RL (cm)	280.79 ± 24.03 **a**	536.47 ± 58.94 **a**	374.38 ± 32.34 **a**	492.72 ± 27.65 **a**
Total RSA (cm^2)	8.47 ± 0.74 **b**	14.15 ± 1.71 **a**	10.81 ± 0.97 **ab**	14.24 ± 0.94 **a**
SRL (m g^{-1})	95.11 ± 5.20 **c**	158.86 ± 10.97 **a**	112.07 ± 2.48 **bc**	131.61 ± 4.93 **ab**
pH	6.97 ± 0.04 **a**	6.54 ± 0.05 **b**	6.90 ± 0.03 **a**	7.03 ± 0.05 **a**

Discussion:

Here, a multi-faceted approach was taken to investigate the effects of a carbonatite on two crop plants, compare these effects to those of calcitic lime, and to briefly explore how carbonatite impacts soil microorganisms. Key experimental findings are summarized in Table 4.6. The response of each plant type to SRC was distinct, although changes for both plants were most often seen in their root architecture. Furthermore, the effects SRC had on plants and soil microorganisms were often very different from those of lime, despite them sharing broad mineralogical similarities (i.e., high calcite content).

Table 4.6: Brief summary of key experimental findings focusing on the effects of the amendment on the simple agroecosystem. R:S = root:shoot, SRL = specific root length, CFU = colony-forming units.

Crop	Experiment	Key result(s)
Pea	**1 - Nodulation**	• Lime and SRC have similar effects on nodulating plants • SRC effects were slightly greater than those of lime
	2 - Early nutrients	• Nutrient addition did not alter early growth aside from slight but significant changes to R:S ratio
	3 - Microbes	• Inoculation increased SRL for SRC-treated plants only • Inoculation did not change CFUs in SRC-treated substrates but decreased them in lime-treated substrates
Wheat	**4 - Development**	• Both trials had consistent patterns, but differences were greater in trial one • Plants with SRC grew more than plants with other amendments • Plants with SRC had the lowest SRL • Plants with SRC had the strongest relationship between SRL and shoot nutrient contents, especially in trial one
	5 - Early nutrients	• SRC amendment effects on seedling growth were similar to those of nutrient addition • Nutrient source (SRC or nutrient solution) dictated response of root parameters

Under the low-nutrient conditions used here, the addition of carbonatite to the growing substrate had strong benefits for wheat. The data suggest that such positive effects were due to the SRC increasing the nutrient content of the substrate, and the wheat responding to the substrate changes. Total SRL was the strongest indicator in support of this conclusion. The SRL of a root system, defined as the ratio between its length and its biomass, has been used as an estimate of the overall diameter of roots (Fitter 1985), and as an indicator of the nutrient foraging strategy for herbaceous plants (Freschet and Roumet 2017; Wen et al. 2019). It has also been shown in some plants, e.g., for wheat but not for pea, to reflect the soil P status (Hill et al. 2006; Wen et al. 2019). For wheat, a higher SRL was found in plants grown in soils with lower P content (20 mg kg^{-1} P versus 200 mg kg^{-1}), and vice versa (Wen et al. 2019). This means that in low nutrient soils wheat increases the length of its root system relative to its root biomass. In this study, a lower SRL was noted for 56 day-old wheat in the presence of the carbonatite than for wheat growing in the absence of the carbonatite. According to the literature, this SRL decrease would correspond to the wheat provided with SRC having thicker roots than those plants without SRC, and that the substrate of plants provided with SRC had more P content (Wen et al. 2019). It is thus hypothesized that the changes in the root architecture reflect the substrate nutrient content and availability resulting from the addition of SRC. Because SRL represents the proportional change in root length and biomass, it provides a clearer picture than that given by the root length alone, i.e., the SRL accounts for root systems having a higher overall length because of their increased size. Additional evidence towards this hypothesis is given by the connection between the SRL and the shoot nutrient contents: with the wheat given SRC, lower SRL values were connected with higher shoot contents of most nutrients. The negative relationship between SRL and nutrient content was much weaker for plants grown with lime or with sand, and in some cases the relationship was positive. While this analysis suggests that SRL can be an indicator of nutrient uptake, further work is needed to elaborate on this phenomenon as the analysis here was limited by the sample size (n = 5-6 per treatment per trial) and the disparity between growing conditions (e.g., between trials one and two). However, it is well established that nutrient availability (or lack thereof) affects root system architecture (e.g., Fitter 1985; Ostonen et al. 2007; Gruber et al. 2013; Freschet and Roumet 2017; Wen et al. 2019).

The altered root morphologies also had cascading effects for plant nutrition that extended beyond those directly attributable to SRC. The relationship between SRL and the shoot content of P and K in plants provided with SRC can be explained by a greater exploitation of the soil volume and a higher weathering of SRC by the thicker plant roots. However, this does not explain the higher shoot N content of these plants, as SRC is known to contain negligible amounts of N (Sage 1987). If this is the case, how did the SRC-treated plants acquire this N? During the 8-week growing period, all wheat plants were provided with an N:P:K fertilizer, and it is hypothesized that the overall larger volume of the soil occupied by the root system of SRC-treated plants allowed them to intercept more of the added N fertilizer than that of the control or lime-treated plant root systems.

The results obtained with wheat seedlings (14 DAP) support the conclusion that benefits to wheat were nutrient-based: young plants responded positively to substrate nutrient addition, and plants given lime plus additional nutrients were similar to plants given only SRC in many ways. Of importance is that for young wheat, the SRL was indicative of the substrate nutrient availability; plants accessing water-soluble nutrients had thicker roots per unit area (lower SRL) than plants accessing carbonatite nutrients which had thinner roots (higher SRL). This result contrasts to what was observed for the older wheat (56 DAP): in the case of SRC-treated plants, the SRL of older plants was almost three times lower than that of younger plants whereas in the case of lime-treated plants, the SRL values were comparable between older and younger plants. Because this change was only observed in the SRC-treated plants, it is hypothesized that the SRL is a function of the weathering status of SRC and subsequent nutrient availability. In the soils of the younger 2 week-old plants, the SRC likely did not have sufficient time to weather and release nutrients, whereas in the older 8 week-old plants, it had been subjected to biotic and abiotic weathering factors for four times as long and it is assumed to have released more nutrients during this time.

Though a clear mechanism is provided to explain how SRC affects wheat growth under these conditions, questions remain about how soil microorganisms may be involved, as SRC did not increase soil respiration in the same manner as lime. Increases in soil CO_2 respiration with lime application is a common finding (Fuentes et al. 2006; Holland et al. 2018) which has been attributed

to the release of CO_2 from $CaCO_3$ breakdown and the neutral substrate pH being more conducive to microbial growth (Fuentes et al. 2006). In light of this, it is unclear why SRC did not have the same effect given it contains $CaCO_3$ and acts on pH in a manner similar to lime. One possibility is an inhibitory role of Mn; for some microorganisms Mn is toxic (Gadd and Griffiths 1977), while others are able to detoxify or make use of this element for metabolic or protective purposes (Ghiorse 1988). It is expected that the soil microorganisms encountered elevated substrate Mn levels from SRC dissolution. Although available substrate Mn was not measured here, evidence towards this conclusion comes from a) the shoot Mn content of SRC-treated wheat which was enriched, b) the uptake of Mn (as Mn^{+2}) by plants is not regulated and is thought instead to depend largely on the rhizosphere pH (Clarkson 1988), and c) soils influenced *in situ* by SRC at the deposit showed elevated Mn contents over those soils which were not influenced (Jones et al. 2019). Because of these reasons, the plant tissue content likely reflected the availability of Mn in the substrate.

For pea, responses to carbonatite addition were more complex and were seen predominantly in the root systems. The boost in nodulation with SRC under low-nutrient conditions observed by Jones (2016) was not seen here, and plants provided with lime nodulated similarly to plants provided with SRC. A lower number of nodules were borne by plants grown in lime- or SRC-amended substrates than by control plants; however, this does not seem to have negatively affected plant or nodulation performance. Because of the similarities between the parameters measured in plants provided with lime and those of plants provided with SRC, the effects of SRC on nodulation seen previously are concluded to largely be caused by the increased substrate pH and lack of substrate nitrogen (plants only had access to SRC and de-ionized water in Jones (2016)). Substrates with low nitrogen levels (Bollman and Vessey 2006) and of neutral pH (Holland et al. 2018) are known to promote nodulation. Additionally, the increase in root system growth seen with SRC (Jones 2016) could not be replicated through the combined used of lime and nutrients, which indicates that other factors are at work.

The results obtained here with pea and agricultural soil microorganisms indicate that pea responds not only to the type of amendment but also to the presence of soil microorganisms. Changes

were seen predominantly in the root systems of plants grown with agricultural microorganisms but were not reflected in the nutrient contents of their shoots. These changes consisted of increases in total root length and surface area which were seen with SRC but not with lime, indicating that the presence or absence of certain microorganisms modulates the response of pea roots to the carbonatite. Unlike what was observed with wheat, the SRL of pea provided with SRC or with lime were similar. This is not surprising, as pea SRL has previously been shown not to respond to differences in substrate nutrient content (Wen et al. 2019). However, the SRL of pea grown in SRC-amended substrates did change depending on whether the plants were inoculated or not with agricultural soil microorganisms. Plants grown in SRC-amended substrates which were not inoculated with agricultural microorganisms had significantly lower SRL values than plants in the other treatments. When inoculated, plants grown in SRC-amended substrates had SRL values similar to those of plants grown in lime-amended substrates. It has been shown in the literature that when pea is inoculated with beneficial microorganisms that assist in P uptake (e.g., *Penicillium balaii*), the total root system SRL increased (Vessey and Heisinger 2001). Because of differences between inoculated and non-inoculated plants grown in SRC-amended substrates, I propose that the carbonatite acts *indirectly* on pea growth by regulating the presence or absence of certain beneficial substrate microorganisms associated with nutrient uptake. This could be caused by SRC acting as a nutrient source for those microorganisms able to weather and release nutrients, e.g., by *Burkholderia* or *Pseudomonas ssp*. (Uroz et al. 2009), or by elements from SRC such as Mn acting to select for those microorganisms able to tolerate them, e.g., Mn tolerance by *Arthrobacter ssp* (Ghiorse (1988). The selective effect of SRC on microorganisms is supported by the CFU count data obtained; while culturable heterotrophic microorganisms dropped significantly after inoculation in lime-treated substrates, the counts in SRC-treated substrates did not change after inoculation. It has already been shown in the soils above the SRC deposit that the soil properties which are influenced by the carbonatite can increase or decrease the abundance of certain microorganisms (Jones et al. 2019; **Chapter 3**). A selective general role of rocks and minerals on soil microorganisms has already been proposed, whereby minerals are considered "inorganic analogues" to roots in terms of their effects on soil microorganisms (Uroz et al. 2015). While this selective role is usually considered to be the result of differences in the ability of

microorganisms to benefit and acquire nutrients from minerals, a selective pressure may also arise when certain elements, such as Mn (Ghiorse 1988), are abundant enough to become toxic to microorganisms. Therefore, while in the case of wheat a simple nutrient-based mechanism can explain the impact of carbonatite on plant growth, in the case of pea the situation is more complex and it seems to involve a three-way interaction between the plant, the carbonatite, and the soil microorganisms. This is supported by previous work showing that pea increases root exudation (acid phosphatases and carboxylases) and colonization by arbuscular mycorrhizal fungi in response to P-limiting conditions to facilitate nutrient uptake (Wen et al. 2019). This is also in contrast to wheat, which responded to P-limiting conditions largely by altering root architecture (Wen et al. 2019). However, more research is needed to clarify these interactions and the potential consequences for plant growth.

Lessons and insights regarding the use of rock fertilizers for agriculture

Perhaps the most important finding from this study is that the context under which carbonatites are used determines their effectiveness in promoting plant growth. While chemical fertilizers are considered broadly applicable to many plant species depending on their nutrient requirements, carbonatite rock fertilizers seem less broadly applicable and their benefits appear to be a function of plant species and growing environment. Based on the findings of Wen et al. (2019), we propose that the effectiveness of a carbonatite to deliver nutrients in a given soil can be quantified through a decrease in total SRL and is directly related to the nutrient foraging strategies of a given plant species. For plants that alter their root morphology to exploit more soil volume (e.g., wheat), it is expected that carbonatites would be highly effective at promoting plant growth in situations where the substrate nutrient content is low (e.g., as in damaged or depleted agricultural soils). For plants that rely on more intensely mining an occupied soil volume through increased exudation and/or partnerships with soil microorganisms, such as pea which partners with nitrogen-fixing rhizobia and mycorrhizal fungi, it is expected that the effectiveness of the carbonatite to promote plant growth will depend on the soil conditions being conducive to these microbial partnerships. In either case, a broad

application of SRC which minimizes high soil densities of the carbonatite will likely prove beneficial, as more clumped distributions are predicted to negatively affect mineral dissolution (Kirk and Nye 1986). It must also be mentioned that elevated Mn contents were observed in both plant species when provided with SRC; due to the potential phytotoxicity of this micro-nutrient (Fernando and Lynch 2015), caution should be exercised when using SRC under conditions where Mn phytotoxicity may occur (e.g., hypoxic soils; Fernando and Lynch 2015). Similar concerns have been noted with Sr in the comparable Stjernøy carbonatite deposit (Myrvang et al. 2017) and high concentrations of undesirable elements may represent a common problem with agricultural carbonatites.

Ultimately, these results show a promising role for carbonatites in agricultural settings as nutrient sources, but more work is needed to characterize their benefits and limitations - not necessarily in terms of their weathering potential like other rock fertilizers, but in terms of how different plant species respond to their presence in agroecosystems and whether the presence of certain elements (e.g., Mn or Sr) hinder or benefit their usefulness. Furthermore, the three-way interaction of carbonatites with soil microorganisms and crop plants, especially those beneficial in agriculture, needs to be assessed.

Literature cited:

Ahmed, E., and Holmström, S.J.M. 2015. Microbe–mineral interactions: The impact of surface attachment on mineral weathering and element selectivity by microorganisms. Chem. Geol. **403**: 13–23. doi:10.1016/j.chemgeo.2015.03.009.

Arcand, M.M., Lynch, D.H., Voroney, R.P., and van Straaten, P. 2010. Residues from a buckwheat (*Fagopyrum esculentum*) green manure crop grown with phosphate rock influence bioavailability of soil phosphorus. Can. J. Soil Sci. **90**(2): 257–266. doi:10.4141/cjss09023.

Bakken, A.K., Gautneb, H., and Myhr, K. 1997a. Plant available potassium in rocks and mine tailings with biotite, nepheline and K-feldspar as K-bearing minerals. Acta Agric. Scand. Sect. B - Soil Plant Sci. **47**(3): 129–134. doi:10.1080/09064719709362452.

Bakken, A.K., Gautneb, H., and Myhr, K. 1997b. The potential of crushed rocks and mine tailings as slow-releasing K fertilizers assessed by intensive cropping with Italian ryegrass in different soil types. Nutr. Cycl. Agroecosystems **47**: 41–48. doi:10.1007/BF01985717.

Bisseling, T., Van Den Bos, R.C., and Van Kammen, A. 1978. The effect of ammonium nitrate on the synthesis of nitrogenase and the concentration of leghemoglobin in pea root nodules induced by *Rhizobium leguminosarum*. Biochim. Biophys. Acta - Gen. Subj. **539**(1): 1–11.

doi:10.1016/0304-4165(78)90115-0.

Bollman, M.I., and Vessey, J.K. 2006. Differential effects of nitrate and ammonium supply on nodule initiation, development, and distribution on roots of pea (Pisum sativum). Can. J. Bot. **84**(6): 893–903. doi:10.1139/b06-027.

Clarkson, D.T. 1988. The uptake and translocation of manganese by plant roots. *In* Manganese in Soils and Plants. Springer Netherlands, Dordrecht. pp. 101–111. doi:10.1007/978-94-009-2817-6_8.

Fernando, D.R., and Lynch, J.P. 2015. Manganese phytotoxicity: new light on an old problem. Ann. Bot. **116**: 313–319. doi:10.1093/aob/mcv111.

Fitter, A. 1985. Functional significance of root morphology and root system architecture. *In* Ecological Interactions in Soil. *Edited by* A. Fitter. Blackwell Scientific Publications, Palo Alto, CA. pp. 87–107.

Food and Agriculture Organization of the United Nations. (n.d.). FAO Ecocrop profile for wheat. Available from http://ecocrop.fao.org/ecocrop/srv/en/dataSheet?id=2114.

Freschet, G.T., and Roumet, C. 2017. Sampling roots to capture plant and soil functions. Funct. Ecol. **31**(8): 1506–1518. doi:10.1111/1365-2435.12883.

Fuentes, J.P., Bezdicek, D.F., Flury, M., Albrecht, S., and Smith, J.L. 2006. Microbial activity affected by lime in a long-term no-till soil. Soil Tillage Res. **88**: 123–131. doi:10.1016/j.still.2005.05.001.

Gadd, G.M., and Griffiths, A.J. 1977. Microorganisms and heavy metal toxicity. Microb. Ecol. **4**(4): 303–317. doi:10.1007/BF02013274.

Ghiorse, W.C. 1988. The biology of manganese transforming microorganisms in soil. *In* Manganese in Soils and Plants. Springer Netherlands, Dordrecht. pp. 75–85. doi:10.1007/978-94-009-2817-6_6.

Goldich, S.S. 1938. A study in rock-weathering. J. Geol. **46**(1): 17–58. doi:10.1086/624619.

Gruber, B.D., Giehl, R.F.H., Friedel, S., and von Wiren, N. 2013. Plasticity of the *Arabidopsis* root system under nutrient deficiencies. Plant Physiol. **163**(1): 161–179. doi:10.1104/pp.113.218453.

Guinel, F.C., and Sloetjes, L.L. 2000. Ethylene is involved in the nodulation phenotype of *Pisum sativum* R50 (*sym16*), a pleiotropic mutant that nodulates poorly and has pale green leaves. J. Exp. Bot. **51**(346): 885–894. doi:10.1093/jexbot/51.346.885.

Gulden, R.H., and Vessey, J.K. 1998. Low concentrations of ammonium inhibit specific nodulation (nodule number g^{-1} root DW) in soybean (*Glycine max* [L.] Merr.). Plant Soil **198**(2): 127–136. doi:10.1023/A:1004281713589.

Hill, J.O., Simpson, R.J., Moore, A.D., and Chapman, D.F. 2006. Morphology and response of roots of pasture species to phosphorus and nitrogen nutrition. Plant Soil **286**(1–2): 7–19. doi:10.1007/s11104-006-0014-3.

Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A., and Hayes, R.C. 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. Sci. Total Environ. **610**–**611**(1): 316–332. doi:10.1016/j.scitotenv.2017.08.020.

Jones, A.P., Genge, M., and Carmody, L. 2013. Carbonate melts and carbonatites. Rev. Mineral. Geochemistry **75**: 289–322. doi:10.2138/rmg.2013.75.10.

Jones, J.M. 2016. Spanish River Carbonatite: Its benefits and potential use as a soil supplement in agriculture. Masters thesis, Wilfrid Laurier University. Available from https://scholars.wlu.ca/etd/1795.

Jones, J.M., Webb, E.A., Lynch, M.D., Charles, T.C., Antunes, P.M., and Guinel, F.C. 2019. Does a carbonatite deposit influence its surrounding ecosystem? FACETS **4**: 389-406. doi:10.1139/facets-2018-0029.

Kirk, G.J.D., and Nye, P.H. 1986. A simple model for predicting the rates of dissolution of sparingly soluble calcium phosphates in soil. II. Applications of the model. J. Soil Sci. **37**(4): 541–554. doi:10.1111/j.1365-2389.1986.tb00386.x.

Lindahl, V., and Bakken, L.R. 1995. Evaluation of methods for extraction of bacteria from soil. FEMS Microbiol. Ecol. **16**: 135–142. doi:10.1016/0168-6496(94)00077-A.

Myrvang, M.B., Heim, M., Krogstad, T., Almås, Å.R., and Gjengedal, E. 2017. The use of carbonatite rock powder as a liming agent. J. Plant Nutr. Soil Sci. **180**: 326–335. doi:10.1002/jpln.201600455.

Myrvang, M.B., Hillersøy, M.H., Heim, M., Bleken, M.A., and Gjengedal, E. 2016. Uptake of macro nutrients, barium, and strontium by vegetation from mineral soils on carbonatite and pyroxenite bedrock at the Lillebukt Alkaline Complex on Stjernøy, Northern Norway. J. Plant Nutr. Soil Sci. **179**(6): 705–716. doi:10.1002/jpln.201600328.

Ogle, D., Wheeler, P. W., and Dinno, A. 2019. FSA: Fisheries Stock Analysis. R package version 0.8.24. Available from https://github.com/droglenc/FSA.

Oono, R., and Denison, R.F. 2010. Comparing symbiotic efficiency between swollen versus nonswollen rhizobial bacteroids. Plant Physiol. **154**(3): 1541–1548. doi:10.1104/pp.110.163436.

Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M.R., Lõhmus, K., Majdi, H., Metcalfe, D., Olsthoorn, A.F.M., Pronk, A., Vanguelova, E., Weih, M., and Brunner, I. 2007. Specific root length as an indicator of environmental change. Plant Biosyst. **141**(3): 426–442. doi:10.1080/11263500701626069.

Profile Products, L. 2015. Turface® product safety data sheet. Available from http://www.turface.com/products/infield-conditioners/mvp [accessed 15 September 2016].

Reddy, M.S., Kumar, S., Babita, K., and Reddy, M.S. 2002. Biosolubilization of poorly soluble rock phosphates by *Aspergillus tubingensis* and *Aspergillus niger*. Bioresour. Technol. **84**(2): 187–189. doi:10.1016/S0960-8524(02)00040-8.

Rowell, D. 1995. The laboratory determination of the respiration rate of the microbial biomass. *In* Soil science: methods and application, 1st edition. Longman Scientific and Technical, Essex. pp. 117–119.

Sage, R.P. 1987. Spanish River Carbonatite Complex. Ontario Ministry of Northern Development and Mines, Toronto, CA.

Sauer, D.B., and Burroughs, R. 1986. Disinfection of seed surfaces with sodium hypochlorite. Phytopathology **76**: 745. doi:10.1094/Phyto-76-745.

Slawek, S. 2013. Vermiculite product safety data sheet. Available from http://www.plantproducts.com/ca/viewproduct.php?pid=117 [accessed 15 September 2016].

Uroz, S., Calvaruso, C., Turpault, M., and Frey-Klett, P. 2009. Mineral weathering by bacteria: ecology, actors and mechanisms. Trends Microbiol. **17**(8): 378–387. doi:10.1016/j.tim.2009.05.004.

Uroz, S., Kelly, L.C., Turpault, M., Lepleux, C., and Frey-Klett, P. 2015. The mineralosphere concept : mineralogical control of the distribution and function of mineral-associated bacterial communities. Trends Microbiol. **23**(12): 751–762. doi:10.1016/j.tim.2015.10.004.

van Straaten, P. 2007. Agrogeology: The use of rocks for crops. Enviroquest Ltd., Toronto, CA.

Vessey, J.K., and Heisinger, K.G. 2001. Effect of *Penicillium bilaii* inoculation and phosphorus fertilisation on root and shoot parameters of field-grown pea. Can. J. Plant Sci. **81**(3): 361–366. doi:10.4141/P00-083.

Vincent, J. 1970. A manual for the practical study of the root-nodule bacteria. Blackwell Scientific Publications, Oxford and Edinburgh.

Watson, M., and Brown, J. 1998. pH and lime requirement. *In* Recommended Chemical Soil Test Procedures for the North Central Region. *Edited by* U.S.D.A. Missouri Agricultural Experiment Station SB 1001.

Wen, Z., Li, H.H., Shen, Q., Tang, X., Xiong, C., Li, H.H., Pang, J., Ryan, M.H., Lambers, H., and Shen, J. 2019. Tradeoffs among root morphology, exudation and mycorrhizal symbioses for phosphorus-acquisition strategies of 16 crop species. New Phytol.: nph.15833. doi:10.1111/nph.15833.

Woolley, A., and Kempe, D. 1989. Carbonatites: nomenclature, average chemical compositions, and element distribution. *In* Carbonatites: Genesis and evolution. *Edited by* Bell K. Unwin Hyman, London. pp. 1–14.

Wu, H., Pratley, J., Lemerle, D., An, M., and Liu, D.L. 2007. Autotoxicity of wheat (*Triticum aestivum* L.) as determined by laboratory bioassays. Plant Soil **296**: 85–93. doi:10.1007/s11104-007-9292-7.

Zadoks, J.C., Chang, T.T., and Konzak, C.F. 1974. A decimal code for the growth stages of cereals. Weed Res. **14**: 415–421. doi:10.1111/j.1365-3180.1974.tb01084.x.

Chapter 4 - Supplemental data

Table S4.1: Crop types and UTM coordinates for mixed agricultural microbiota inoculum. Soils were collected on Oct 14/2017, and stored at 4°C until use.

Crop type	**Location**	**UTM coordinates**
Alfalfa	Waterloo/St. Jacobs	17T 0535686 4818208
Clover (cover)	Waterloo/St. Jacobs	17T 0534186 4818506
Wheat w/ old soybean	Waterloo/St. Jacobs	17T 0535838 4817287
Corn	Waterloo/St. Jacobs	17T 0536582 4817576
Corn	Waterloo/St. Jacobs	17T 0537840 4816440
Vineyard soil	Beamsville, ON	17R 3340032 652172
Simcoe Research Station	Simcoe Research Station	17T 559541 4745215

Table S4.2: Ambient greenhouse conditions under which the 56 day (a) and 14 day (b) wheat experiments were conducted, separated by trial and experiment.

a) Experiment 4

Grown: Nov 22/2017-Jan 17/2018	**Trial 1:**
Temperature (°C)	18.8
Relative humidity (%)	34.7
Photosynthetically-active radiation (µmol)	116.2
Outdoor Light Energy (W/m²)	50.4
*Grown Mar 18/2018-May 13/2018**	**Trial 2:**
Temperature (°C)	20.9
Relative humidity (%)	34.0
Photosynthetically-active radiation (µmol)	163.4
Outdoor Light Energy (W/m²)	188.3

* data unavailable for Apr 2018

b) Experiment 5

Grown June 6/2018-June 20/2018	**Trial 1:**
Temperature (°C)	23.0
Relative humidity (%)	54.1
Photosynthetically-active radiation (µmol)	150.6
Outdoor Light Energy (W/m²)	243.5
Grown Oct 16/2018-Oct30/2018	**Trial 2:**
Temperature (°C)	NA
Relative humidity (%)	NA
Photosynthetically-active radiation (µmol)	NA
Outdoor Light Energy (W/m²)	NA

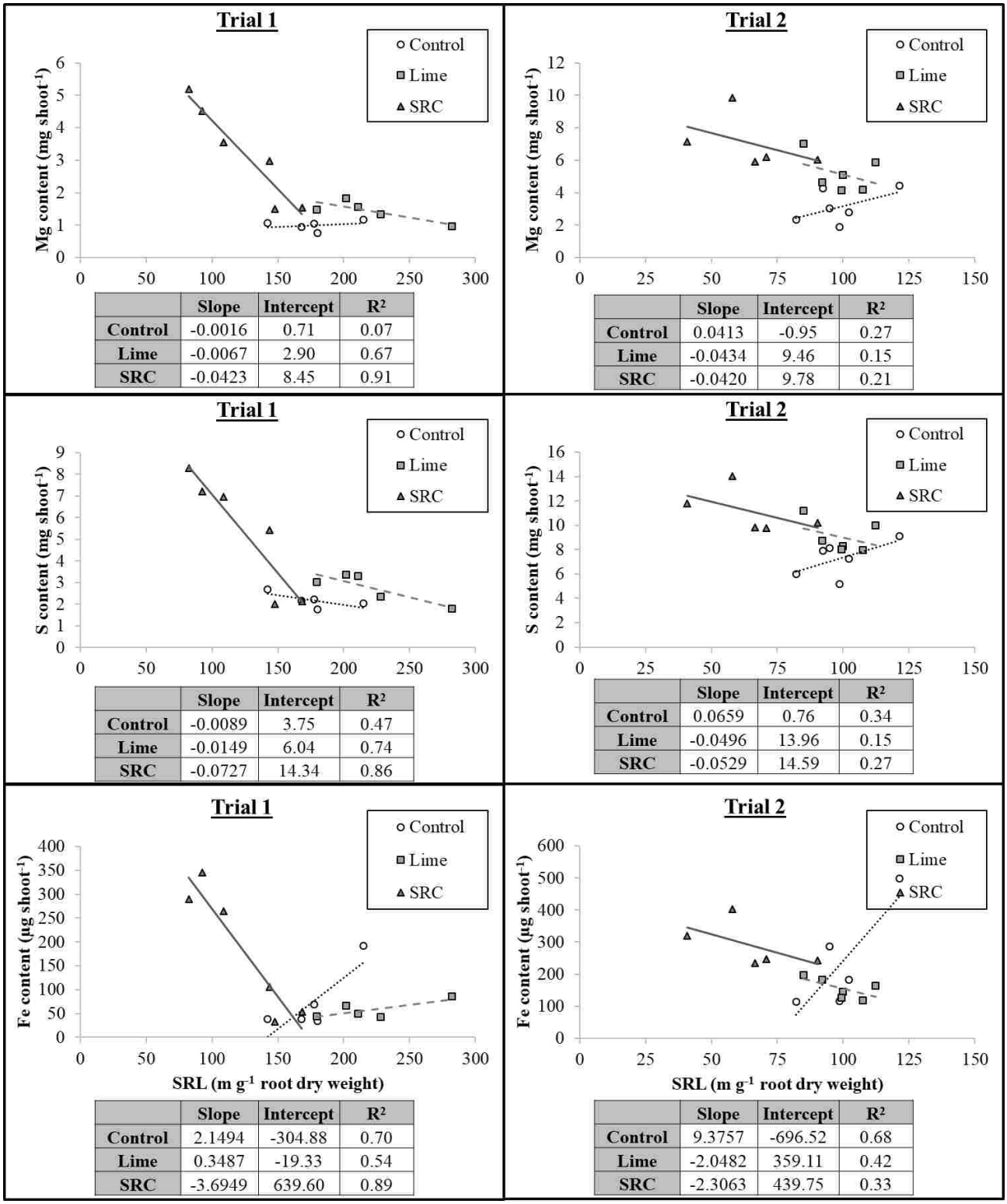
*data unavailable for Oct 2018

Table S4.3: Yield of wheat plants (n = 4-5 per treatment) from trial one grown until senescence in either 1:10 silica sand:substrate (control), 1:15 calcitic lime:substrate (lime), or 1:10 SRC:substrate (SRC). Significant differences in biomass are indicated by different letters (Kruskal-Wallis + post-hoc Dunn's test at 95% confidence level).

	Seeds	**Seed DW (g)**
Control	92.5 ± 9.8 **b**	3.3098 ± 0.3074 **ab**
Lime	86.0 ± 8.3 **b**	2.8040 ± 0.3210 **b**
SRC	140.2 ± 13.0 **a**	4.9945 ± 0.5199 **a**

Table S4.4: Biomass, growth, and root architecture parameters of 14 day-old wheat (n = 9-10 plants per treatment) from trial two grown in substrates amended with lime or SRC (LC and SC), or substrates amended and prepared with additional nutrients (LH for lime Hoagland and SH for SRC Hoagland). Significant differences are indicated by different letters (Kruskal-Wallis + post-hoc Dunn's test at 95% confidence level). DW = dry weight, GR = growth rate, RL = root length, RSA = root surface area, SRL = specific root length.

	LC	**SC**	**LH**	**SH**
Shoot DW (mg)	20.43 ± 1.03 **b**	27.34 ± 3.27 **ab**	28.65 ± 3.02 **ab**	38.82 ± 2.38 **a**
Root DW (mg)	17.17 ± 1.68 **a**	17.97 ± 2.45 **a**	18.45 ± 2.05 **a**	20.90 ± 1.44 **a**
GR (cm day^{-1})	1.23 ± 0.05 **b**	1.59 ± 0.11 **ab**	1.66 ± 0.09 **a**	1.97 ± 0.07 **a**
Total RL (cm)	273.11 ± 10.18 **b**	419.53 ± 41.89 **a**	421.25 ± 34.97 **a**	452.04 ± 29.90 **a**
Total RSA (cm^2)	12.10 ± 0.39 **b**	18.91 ± 2.02 **a**	18.17 ± 1.64 **a**	22.02 ± 1.44 **a**
SRL (m g^{-1})	164.03 ± 9.62 **b**	246.10 ± 13.32 **a**	234.25 ± 7.76 **a**	219.89 ± 10.89 **a**
pH	7.58 ± 0.05 **a**	7.43 ± 0.07 **a**	7.57 ± 0.03 **a**	7.41 ± 0.04 **a**



	Slope	Intercept	R^2
Control	-0.0016	0.71	0.07
Lime	-0.0067	2.90	0.67
SRC	-0.0423	8.45	0.91

	Slope	Intercept	R^2
Control	0.0413	-0.95	0.27
Lime	-0.0434	9.46	0.15
SRC	-0.0420	9.78	0.21

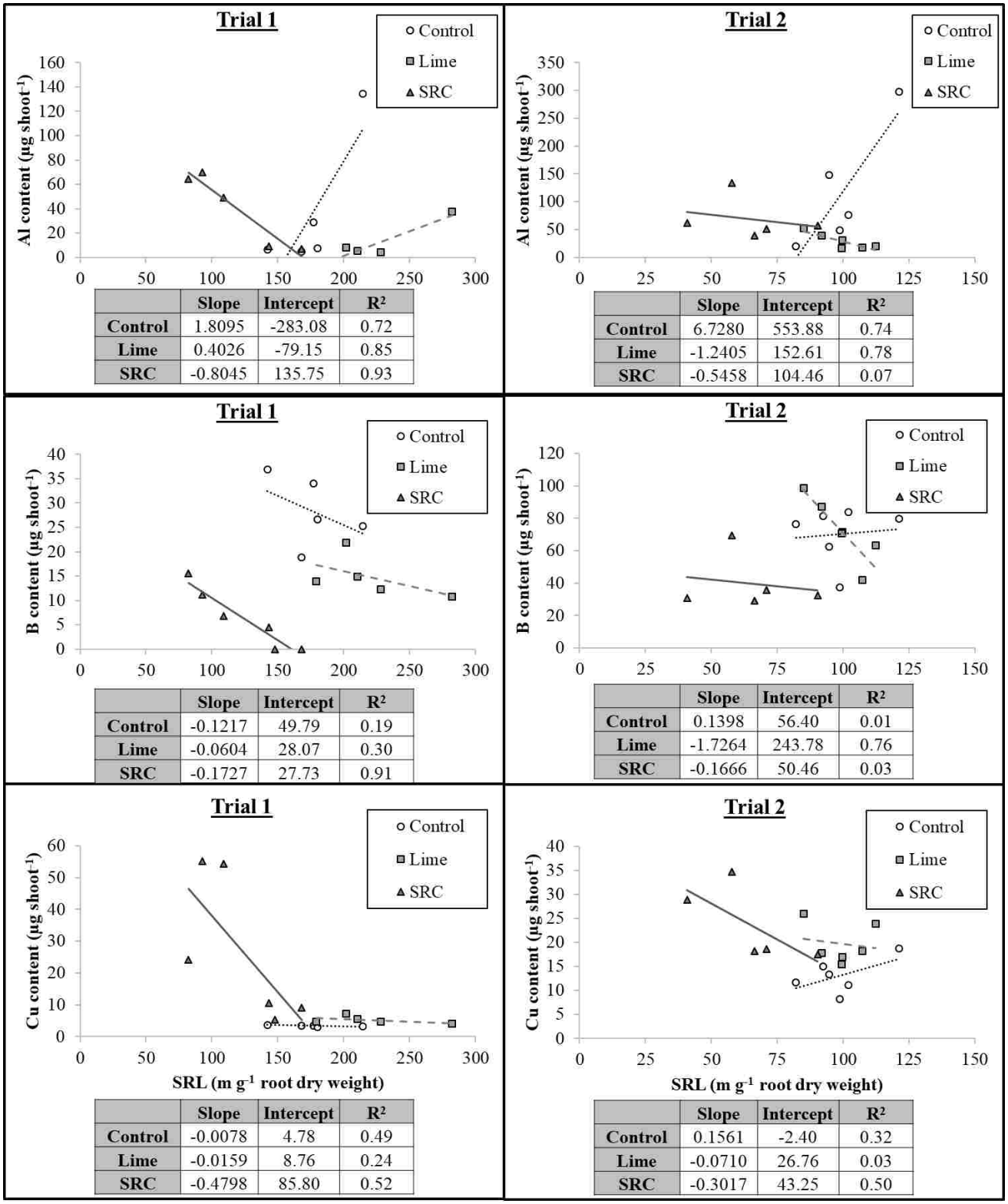
	Slope	Intercept	R^2
Control	-0.0089	3.75	0.47
Lime	-0.0149	6.04	0.74
SRC	-0.0727	14.34	0.86

	Slope	Intercept	R^2
Control	0.0659	0.76	0.34
Lime	-0.0496	13.96	0.15
SRC	-0.0529	14.59	0.27

	Slope	Intercept	R^2
Control	2.1494	-304.88	0.70
Lime	0.3487	-19.33	0.54
SRC	-3.6949	639.60	0.89

	Slope	Intercept	R^2
Control	9.3757	-696.52	0.68
Lime	-2.0482	359.11	0.42
SRC	-2.3063	439.75	0.33

Figure S4.1



	Slope	Intercept	R^2
Control	1.8095	-283.08	0.72
Lime	0.4026	-79.15	0.85
SRC	-0.8045	135.75	0.93

	Slope	Intercept	R^2
Control	6.7280	553.88	0.74
Lime	-1.2405	152.61	0.78
SRC	-0.5458	104.46	0.07

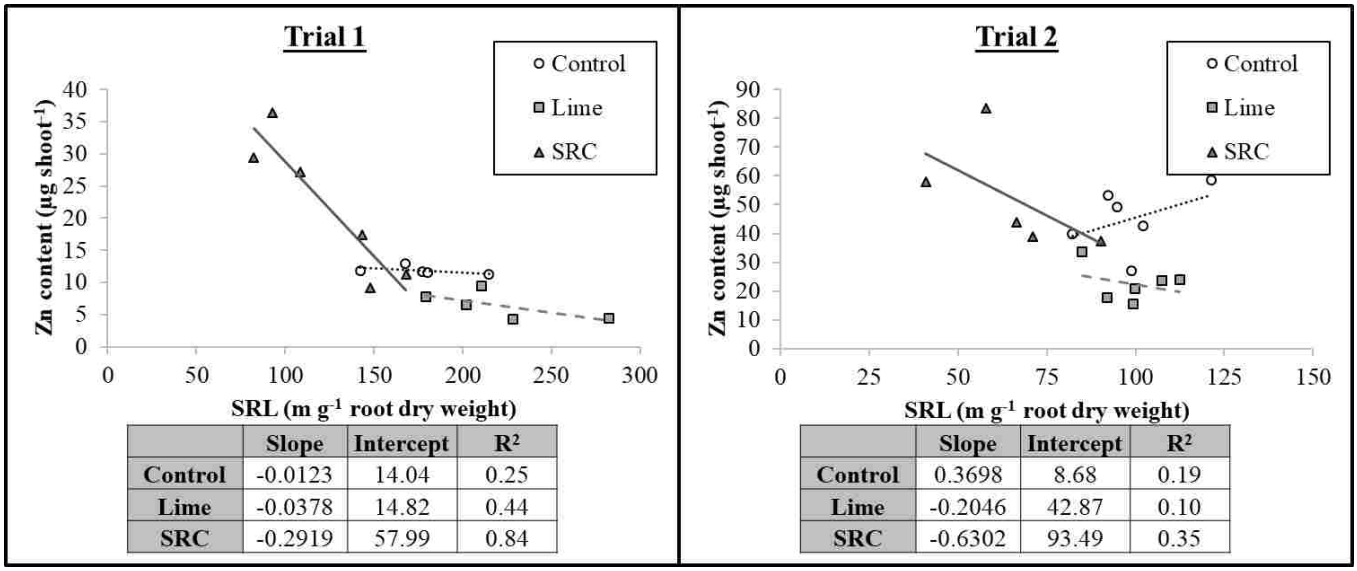
	Slope	Intercept	R^2
Control	-0.1217	49.79	0.19
Lime	-0.0604	28.07	0.30
SRC	-0.1727	27.73	0.91

	Slope	Intercept	R^2
Control	0.1398	56.40	0.01
Lime	-1.7264	243.78	0.76
SRC	-0.1666	50.46	0.03

	Slope	Intercept	R^2
Control	-0.0078	4.78	0.49
Lime	-0.0159	8.76	0.24
SRC	-0.4798	85.80	0.52

	Slope	Intercept	R^2
Control	0.1561	-2.40	0.32
Lime	-0.0710	26.76	0.03
SRC	-0.3017	43.25	0.50

Figure S4.1 continued



	Slope	Intercept	R^2
Control	-0.0123	14.04	0.25
Lime	-0.0378	14.82	0.44
SRC	-0.2919	57.99	0.84

	Slope	Intercept	R^2
Control	0.3698	8.68	0.19
Lime	-0.2046	42.87	0.10
SRC	-0.6302	93.49	0.35

Figure S4.1 continued: Linear regression analysis of specific root length (SRL) and shoot nutrient content in 56 day-old wheat plants (n = 5 per treatment group per trial) grown in either 1:10 silica sand (control), 1:15 calcitic lime:substrate (lime), or 1:10 SRC:substrate (SRC). Linear regression values were derived using the 'lm' function (R software suite version 3.5.2).

Chapter 5 - Carbonatite mechanism of action and context-dependence

This chapter provides a model for the mechanism of action by which SRC impacts soils, plants, and soil microorganisms based on the research findings from the dissertation, and drawing upon current literature. It will not form a stand-alone publication, but will be combined with the literature review on carbonatites.

Carbonatites as rock fertilizers: mechanism of action and context-dependence

The aim of this dissertation was to present an exploration of the carbonatite SRC in terms of its effects on plants, soils, and microorganisms, and to provide a stepping stone for future research into similar rock fertilizers. This exploration was undertaken using a multifold approach: 1) by surveying the SRC deposit and ecosystem overlying the area to quantify the effects of SRC *in situ*, 2) by characterizing the effects SRC has on plants and comparing those against the effects obtained with agricultural lime, and 3) by identifying factors which are affected by SRC and which affect SRC in agricultural ecosystems ("agroecosystems"). The research findings are summarized below. The results are then placed into a descriptive model which illustrates the effects SRC is expected to have at the level of the ecosystem, at the level of the plant, and at the level of soil microorganisms. Finally, the importance of a multidisciplinary, systems-level approach to this and similar research is covered in order to fulfill the program requirements.

General summary of research findings

For the survey project, it was predicted that there would be a signature in the measured ecosystem parameters that would reflect the variable mineralogy within the zones of the SRC deposit. Due to the glacial till overlying the area, the distinct zones could not be separated based on observed ecosystem differences, and only the presence or absence of SRC influence could be ascertained. This

influence was not zone-specific, and while some zones showed moderate SRC influence, others showed none. The soils, plant communities, and microorganism abundances each showed a separate response to the SRC.

In order to interpret the effects of the carbonatite at the ecosystem level, one must consider the landscape situation. While the deposit represents a relatively large area (~ 1.5 km by 2.0 km), it is located within a large forest ecosystem. In the study area outside of the deposit, the soils were slightly acidic (~pH 5), and had a characteristic nutrient profile with specific Ca, Mn, and Al concentrations. Across the deposit, certain sites were found to have soil characteristics that set them apart from the surrounding forest soils: they were more basic (~pH 6), had higher concentrations of Ca and Mn, and lower levels of Al. At the quarry, similar characteristics were seen in the exposed carbonatite, though the values of these parameters here were much higher than elsewhere. The similarities between the exposed carbonatite and some of the sampled soils led us to conclude that despite being somewhat isolated from the surface by a layer of glacial till overburden, the deposit was having an influence on the soil properties in some areas.

Both plants and soil microorganisms responded to the presence of the carbonatite. The plant communities in soils that had been identified as carbonatite-influenced were somewhat different from those in non-influenced soils, with the primary difference being the presence of more ruderal species (e.g., *Acer spicatum* Lamarck and *Solidago altissima* Linnaeus) in carbonatite soils. The changes in plant communities were interpreted as a reflection of a continual “disturbance” in soil properties caused by the presence of the carbonatite relative to the unaffected soils. Plants with broader habitat tolerances could then take advantage of a new niche created in the soils with the altered soil properties. Because of the size of the surrounding forest relative to that of the deposit, these plants were not novel or unusual species, but simply a subset of those already found within this ecosystem. In a sense, the carbonatite was acting as a selective pressure to restrict which plant species were able to thrive in these soils, and this is borne out in the low Shannon diversity indices in the carbonatite-influenced sites. Although the microbial communities as a whole were resilient to the soil changes, twenty-six bacterial OTUs and four fungal OTUs were found to be less abundant in carbonatite-

influenced soils. Two bacterial OTUs, however, were found to be more abundant in carbonatite soils: a *Gaiella occulta* OTU and an *Arthrobacter* (or *Pseudoarthrobacter*) OTU. Thus, as with the plants, the carbonatite acted to restrict the growth of certain organisms and promote the growth of others. It must be noted that because plants can have a strong influence on the soil microbial populations (e.g., Fierer et al. 2005; Zhou et al. 2017) and vice versa (e.g., Calvaruso et al. 2006; Philippot et al. 2013; Burghelea et al. 2015), it would be difficult *in situ* to isolate the effects of the carbonatite on each of these components individually. The interconnectedness between the minerals of the carbonatite rock, the plants, and the microorganisms of the substrate is an important theme which was seen throughout the work completed here.

While it was difficult to untangle the specific effects and mechanisms of the carbonatite on plants and soil microorganisms at the level of the deposit, this was achievable through greenhouse and growth-room experiments where conditions can largely be controlled. Here, the impacts of SRC on wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.) were assessed at the level of individual plants. Additionally, because two of the main drivers of deposit-level changes were increases in soil pH and in Ca concentration, the carbonatite was compared against a calcitic lime to determine whether responses were simply pH-based or were specific to SRC. Because SRC contains a wide variety of minerals while lime contains just one, it was expected that these two amendments would produce different effects on plants and microorganisms. While both SRC and lime increased the pH of the substrate for both plant species, there were indeed differences in plant and microorganism responses between amendments. For wheat grown in an acidic, low-nutrient substrate and provided with a 22:14:6 N:P:K fertilizer, the incorporation of SRC into the substrate strongly promoted plant growth compared to the incorporation of lime or silica sand. Notably, these effects were observed as early as 14 days after planting, indicating that the carbonatite was likely weathering and releasing nutrients in that time frame. The benefits to plant growth were attributed to SRC increasing the substrate-nutrient content, and the ability of the wheat root systems to exploit this increase to take up available nutrients. Despite the significant increases in plant growth, the microbial activity (as measured via respiration of CO_2 from soils) was not affected by carbonatite and was only boosted by

treatment with lime. By considering the shoot nutrient contents, the specific root length (m g^{-1} root dry weight), and the similarities between young (< 14 days) plants treated with a combination of lime and nutrients or with SRC, it was concluded that the carbonatite promotes wheat growth by serving as an efficient source of nutrients. No specific effect of soil microorganisms on plant growth could be discerned during these experiments, but the microbial respiration results suggest that there was interaction between the carbonatite and microorganisms. The breakdown of $CaCO_3$ in lime is known to benefit the activity of microorganisms, especially bacteria, by changing the substrate pH and making the soil environment more conducive to their growth (Fuentes et al. 2006). While SRC also contains $CaCO_3$ (Sage 1987), it was observed that lime increased the microbial respiration but SRC did not. This may be because SRC also increased the substrate Mn and thereby modulated microbial populations (Ghiorse 1988), although this needs to be clarified. It also remains unclear to what extent the microorganism-carbonatite interactions influenced plant growth.

For pea grown in the same acidic, low-nutrient substrate and provided with a full nutrient solution, carbonatite responses were more difficult to distinguish and seemed to depend predominantly on microorganisms. The effect of SRC on nodulation seen by Jones (2016) was clarified to be the result of an increase in pH and the absence of added nitrogen. The use of lime or SRC and additional nutrients produced no discernible effect on seedling peas (<14 DAP) beyond slight changes to the R:S ratio. Differences between lime and SRC for pea were revealed by inoculation with the agricultural soil microorganisms. For instance, the SRL of plants grown in SRC-amended substrates increased with inoculation, whereas inoculation had no effect on plants that were grown in lime-amended substrates. The amendment also interacted strongly with the inoculation to affect the number of heterotrophic microorganisms that could be cultured from the substrates: with lime, only 1/4 of the number of colony-forming-units was obtained from inoculated substrates compared to those not inoculated, while for SRC the inoculation had no effect. Like at the deposit, the carbonatite seemed to act in a stabilizing or selective manner to influence the soil microbial population, and even the addition of a different microbial community did not alter the proportion of culturable heterotrophic bacteria. When the results obtained with wheat and pea are integrated, it is

clear that SRC interacts with the substrate microorganisms in some capacity and that this interaction does produce discernable effects on plant growth. Furthermore, it can be concluded that the growing context (e.g., nutrient regime and/or microorganism presence/absence) and plant type are key drivers of whether a carbonatite can be considered effective as a rock fertilizer.

Mechanism of action for SRCs effects: A model

In interpreting the overall results obtained during the dissertation, a hierarchical approach will be used to model how SRC fits into and interacts with the various components of a given agroecosystem. Areas where further research is needed will also be highlighted. Before the model is presented, a few definitions are required. **Agroecosystems** are ecosystems that are under human management to produce agricultural products (Conway 1987; Gleissman 2004). An agroecosystem can be considered as made up of **holons -** components that are simultaneously individual parts but also part of a whole (Bland and Bell 2007). For example, a plant can be considered individually or as part of the larger plant community. Finally, in **hierarchical models**, the upper level(s) or components set the context for the lower levels (Allen and Starr 2017). Note that here the soil, the plant, and the microorganism are the holons of interest. The overall changes SRC is expected to cause in a given agroecosystem are summarized in Figure 5.1, and each level will covered from the agroecosystem to the rhizosphere.

Changes at the highest level - the agroecosystem

At the agroecosystem level (Figure 5.1a), dissolution of SRC is expected to (at least) increase the soil pH, cation exchange capacity (CEC), Ca content, Mn content, and decrease Al content. This is based upon the survey completed at the SRC deposit. However, at this level there remains a number of unresolved questions:

a) Does SRC increase the content of other nutrients besides Ca and Mn?

b) Does SRC affect the emission of greenhouse gasses from soils (e.g., CO_2, NO_x, CH_4)?

c) Does SRC contribute to or decrease the runoff of highly-mobile nutrients from soils?

d) Does SRC alter the accumulation of organic matter (OM)?

e) Does SRC change the soil structure (e.g., aggregate number or stability)?

The answers to these questions will greatly influence whether SRC, and other carbonatites, can have reliable practical applications as rock fertilizers in agriculture. The answers to questions a) and b) can be estimated based on available information.

a) At the deposit, only Ca and Mn were found to be enriched in carbonatite-influenced soils, implying that when used in an agricultural context only these two elements might be provided to plants. However, the results from Jones (2016) demonstrated that pea could be successfully grown in a low-nutrient substrate with only the carbonatite and N fixed by rhizobia as nutrient sources, suggesting that a variety of nutrients were provided by SRC. Though no data were collected on plant nutrient content at the SRC deposit, nutrient enrichment (of Ca, Mg, K, P; Myrvang et al. 2016) and increased growth (Vestin et al. 2013) of vegetation growing on carbonatite deposits have been previously reported. Thus, it would be surprising that SRC did not increase the content of nutrients besides Ca and Mn in plants when SRC is added to soils assuming conditions are favourable for mineral weathering.

b) During the investigation of the impact of SRC on wheat growth (**Chapter 4**), the amount of CO_2 released from the substrates was used as a measure for microbial respiration. Substrates amended with lime had higher CO_2 levels than the control substrates, as expected based on the literature (e.g., Fuentes et al. 2006). The SRC-amended substrates, however, did not show any increase in CO_2 levels and were comparable to the control substrates. Indirectly, this measurement gives some insight into how SRC could affect CO_2 emissions. Depending on soil dynamics and management practices (e.g., microbial activity and tillage), agricultural soils can be sources or sinks of CO_2 (Paustian et al. 1997, 2000). If SRC is demonstrated to lower microbial respiration, it could become an important tool for

lowering soil CO_2 emissions and increasing soil OM content via depression of microbial-mediated OM breakdown. Also, the nitrification-denitrification balance in a given soil determines the emission of nitrogen-based greenhouse gasses and leaching of nitrogen (Mkhabela et al. 2008), and the impact of SRC on this balance remains to be quantified. As SRC introduces a large quantity of Ca into the soils in a manner similar to lime, it may have similar effects to lime on soil stability. In short time frames (1-3 months), lime has been shown to decrease the soil particle size by disrupting Al bonds in clay minerals, but over long time frames (>6 months), it has been shown to be beneficial due to secondary mineral formation and increased plant growth (Haynes and Naidu 1998). Additionally, since it appears that Mn is introduced with SRC, its usage may affect Mn cycling in soils systems – e.g., by providing more Mn for use in organic matter breakdown by fungi (Keiluweit et al. 2015) or by altering long-term production of humic compounds by Mn oxides (Shindo and Huang 1982; Huang et al. 2005). Thus, how SRC affects the dynamics of soils, e.g., by altering CO_2 emissions, OM sequestration or breakdown, and soil stability, is an important avenue for future research. Finally, it is expected that SRC will have negligible effects in soils which are already high in Ca and/or pH, and this has been partly shown in a study by Arcand et al. (2010) during an investigation of the efficacy of various phosphate rocks to deliver P. At the uppermost level of hierarchy, the soil chemistry changes are expected to frame how plants and microorganisms respond to SRC.

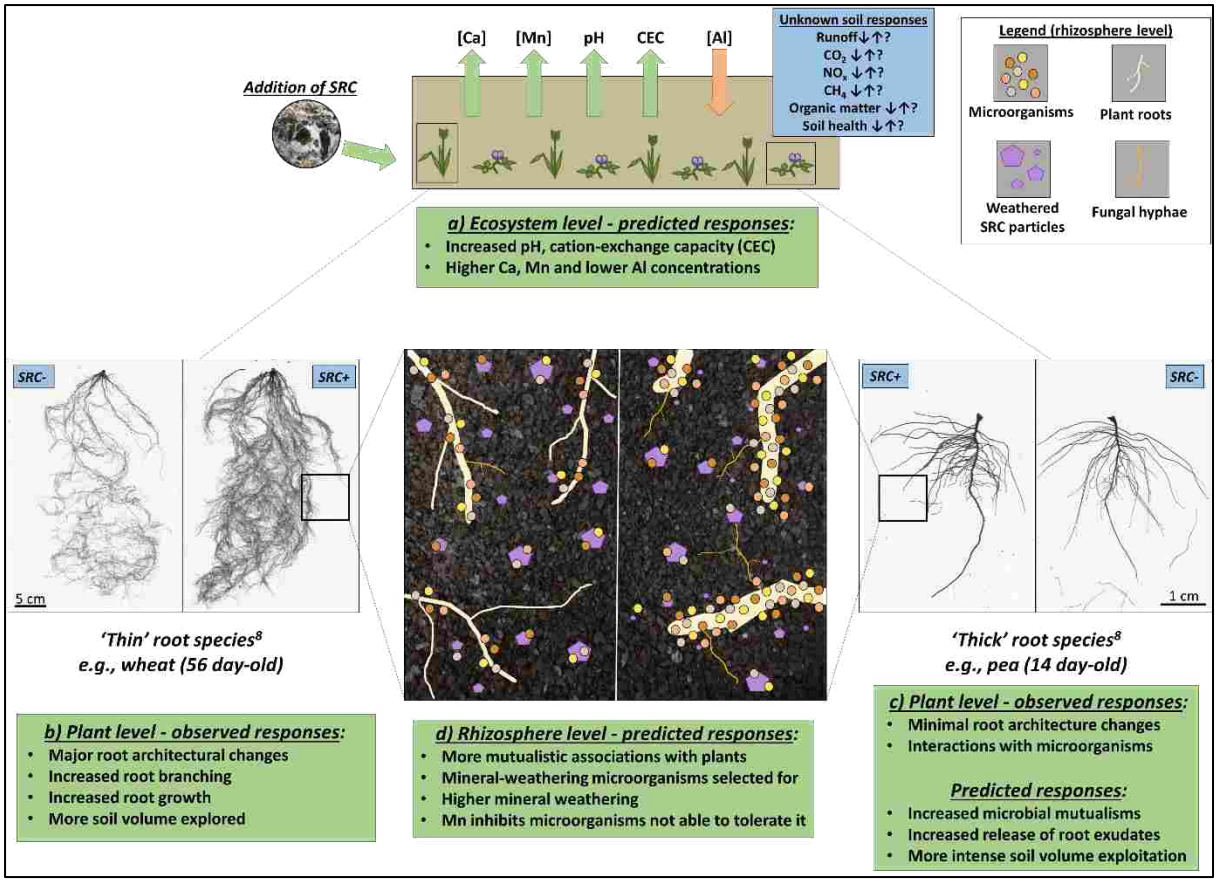


Figure 5.1: Working model of how SRC (or other carbonatites) is expected to alter agroecosystems. At the field level (a), a number of soil chemical changes will occur, and there remain several questions (e.g., will SRC affect the sequestration of organic matter?). At the individual plant level, changes will be a function of plant type, and are predicted to broadly follow one of two paths depending on the nutrient foraging strategy of the plant: thin root exploration (b) or thick root mining (c). At the microorganism level (d), changes are harder to anticipate. It is predicted that SRC incorporation into the soil will increase microbial mutualisms with plants (via cooperative mineral weathering), and that microbial community structures will be altered because of the presence of elements like Mn.

Changes at the level of individual plants

At the level of individual plants (Figure 5.1b, c), SRC is expected to have a number of impacts depending on the growing context. Because the effects that it has on plants are at least partly nutrient-based, SRC is not expected to be beneficial in already fertile soils. There are some experimental data to support this hypothesis from work conducted in 2013-2014 on the application of SRC in an organic greenhouse setting with mini-cucumbers (personal communication, Frédérique Guinel, 2019). There, no discernable changes to plant growth were observed, and this was attributed to the high nutrient content and robustness of the soils prior to SRC application (personal communication, Frédérique Guinel, 2019). The less fertile the soil, the more the impact SRC will have on soils and on plant responses. However, plants responses will be a function of the plant type and associated growing patterns.

As shown in Chapter 4, the nutrient foraging strategy, as exemplified in the root thickness and SRL, appears to be a key driver of plant responses to SRC. Plants with thin roots (Figure 5.1b) that increase the volume of soil explored to gain more nutrients (e.g., wheat) should benefit strongly from SRC application. This could change depending on the distribution of SRC within the substrate: a random distribution of phosphate rocks is expected to give the highest dissolution rates (Kirk and Nye 1986), and thus the more soil volume a root system explores the more SRC it can exploit. While each SRC particle may not be highly weathered, this is compensated for by the high number of particles encountered and acted upon by the root system (Figure 5.1b). While the interaction between these roots and microorganisms is no doubt meaningful, it is currently unclear to what extent plant growth and nutrient availability may be affected by it.

For plants with thicker root systems (e.g., pea), the situation is more complex, but insights may still be gained through the lens of nutrient-foraging strategies (Figure 5.1c). Pea specifically increases exudation patterns and partners with mutualistic soil microorganisms in situations of low nutrient availability (Lodwig et al. 2003; Carbonnel and Gutjahr 2014; Wen et al. 2019), and it is hypothesized that this modulates whether or not pea responds to SRC addition. Data acquired here

and from previous work support this hypothesis. In chapter 4, responses of pea to SRC addition depended on whether plants had been inoculated with soil microorganisms, implying that the microorganisms were partly controlling the response of pea to SRC. Additionally, SRC seemed to have a selective or stabilizing pressure on the microorganisms, as the number of culturable heterotrophs obtained in SRC-amended substrates was the same regardless of whether they had been inoculated or not with the agricultural microbial solution. Thus, plants with thicker roots are expected to produce more root exudates than those plants with thin roots, and have a stronger partnership with soil microorganisms (Figure 5.1c). While fewer SRC particles may be encountered by a plant with thick roots, those particles that are encountered will likely be highly weathered due to the combined action of plant and microorganisms (Figure 5.1c). Partnerships with mycorrhizal fungi may prove to be especially important here, as these fungi are considered to extend the reach of plant root systems, solubilize P, and transfer P to plants (Friese and Allen 1991; Smith et al. 2001; Kernaghan 2005; Kiers et al. 2011; Zhang et al. 2016). Care will have to be taken when carbonatites are used for thick root plants, as the results will likely depend on the impacts of the carbonatite on plants and microorganisms, and also the interactions between the three components. Weathering of the mineral from plant-specific action is also expected and adds complexity. For example, the type of nitrogen provided to soybean (*Glycine max* (L.) Merr.) has been shown to influence whether the plants acidifies or alkalizes its rhizosphere, which subsequently affected the dissolution of phosphate rocks (Aguilar and van Diest 1981).

Changes at the level of the rhizosphere

At the rhizosphere level (Figure 4.6d), the impacts of SRC on microorganisms are harder to predict, and are at this point largely speculative. Results obtained from the survey of the SRC deposit indicate that SRC will to some extent dictate microorganism diversity (Table 3.2). In the substrate of wheat in the greenhouse, microbial respiration was not increased by SRC addition but was increased by lime (Table 4.4). In the substrate of pea in the growth room, the number of culturable heterotrophic

microorganisms was unaffected by inoculation with agricultural microorganisms (Figure 4.6b). While a specific mechanism to explain these cannot be presented here, two possibilities which are not mutually-exclusive can be put forward:

a) Mn (or other elements) from SRC selects for those microorganisms able to tolerate them (e.g., Mn tolerance in *Arthrobacter spp.*; Ghiorse 1988)

b) The nutrients in SRC select for those microorganisms which are able to release them from the minerals. Microorganisms have been shown to weather minerals by exuding compounds such as organic acids (Bennett et al. 2001; Rogers and Bennett 2004; Whitman et al. 2018)

More generally, SRC is likely to act similarly to lime in promoting bacterial abundance through alleviation of acidic soil conditions and the production of a more conducive soil environment (Fuentes et al. 2006). However, increased Mn levels were found not only in the influenced soils at the deposit but also in the shoots of plants grown in SRC-amended substrates, indicating that this element will likely be present in a non-negligible quantity when substrates are amended with SRC. Given that, the benefits from the liming effect may be precluded by the restriction of microorganism growth by Mn from SRC (Figure 5.1d). This could explain why soil respiration was not increased with SRC but was with lime despite the similarity in substrate pH (Table 4.4).

Additionally, the presence of plants is expected to have a strong impact on microbial responses to SRC. Soil microbial populations are most often C-limited (Demoling et al. 2007), and plant roots are a large source of soil C (Helal and Sauerbeck 1986). Therefore, when the rhizosphere and mineralosphere (e.g., around SRC) intersect, strong benefits will likely be realized by those microorganisms able to take advantage of conditions imposed by root and mineral. This is expected to have positive effects on the dissolution of SRC, and such a “mutualism” has already been proposed to explain mineral weathering (Banfield et al. 1999). In brief, a positive feedback loop between microorganisms, roots, and SRC is expected. As microorganisms and plant roots weather SRC, more nutrients are taken up by both sets of microorganisms which would encourage their growth. The proliferation of roots due to higher local nutrient availability would also increase the amount of

carbon available to microorganisms, which would further promote their growth and their ability to weather minerals. Whether this positive feedback "mutualism" can be effectively harnessed to promote weathering of SRC for plant benefits or is already occurring (e.g., with wheat) remains to be seen. The contributions of several microbial genera may prove to be key, for instance *Arthrobacter ssp*. with their tolerance to Mn and utilization of diverse carbon/nitrogen sources (Hagedorn and Holt 1975; Hungate et al. 1987; Ghiorse 1988), *Pseudomonas ssp.* with their known ability to weather minerals and which are enriched in plant rhizospheres (Grayston et al. 1998; Uroz et al. 2007, 2009), and *Burkholderia ssp.* for their weathering effects on several different mineral types (Uroz et al. 2009).

Conclusions, and avenues for future research

While the work presented here represents a substantial advance in our basic understanding of how SRC (or other carbonatites) can affect plants, soils, and soil microorganisms, many questions remain. Foremost of these is how SRC can affect the agroecosystem as a whole (e.g., by moderating soil structure changes or greenhouse gas emissions). Another key question is how SRC exerts its apparent selective pressure on soil microorganisms, as the answer will provide information on how SRC will alter microbially-mediated soil processes like nitrification or respiration. One avenue that I was not able to explore was how SRC fits into an integrated nutrient strategy. An example of this would be the combined use of SRC and compost. Using a low-grade phosphate rock with composts was reported to enhance the availability of P from the rock (Biswas and Narayanasamy 2006). The use of SRC and compost is expected to have similar advantages. Because there will not be a single solution to achieve sustainable agriculture, it will be crucial to test the efficacy of SRC with other management strategies, such as its combined use with cover crops (Christie 2019; VanVolkenburg 2019).

Benefits from carbonatites (and likely other rock fertilizers) will only be realized if a systems-level approach is taken. At the most basic level, mineral weathering is influenced not only by the

chemistry of the soil solution and by the climate (e.g., temperature) but also by the actions of plants and microorganisms. The concept of holons is important here, as plants and microorganisms not only affect but are also affected by changes to the agroecosystem as a whole. Because none of these components work alone, each should be considered in order to gain a fuller perspective of the system (Figure 5.1). The nature of systems biology necessitates a multidisciplinary approach, and this is the approach I took here with the simple agroecosystem (i.e., just soils, plants, and soil microorganisms). A number of different disciplines were utilized (Figure 5.2); each discipline added a different perspective to the research. Only by integrating the complementary component perspectives could a better understanding of the whole system be gained. Effective studies involving rock fertilizers will likewise necessitate the incorporation of several disciplines, not least of all plant physiology and nutrition, geology, soil chemistry, plant and microbial biochemistry, soil microbial ecology, and systems science.

While this work increases our understanding of carbonatites and their impacts in agroecosystems, its significance extends beyond a research setting. Local farmers have shown a strong interest in carbonatites and other rock fertilizers, as was seen during two sustainable agriculture workshops held at Wilfrid Laurier University (17 Feb. 2015 and 20 Feb. 2018). In future studies with carbonatites, emphasis should be placed on working with local agriculturalists to conduct research so that both parties can benefit directly from the findings. Such partnerships also allow a two-way exchange of information with farmers being able to take advantage of the scientific perspectives of researchers, and researchers being able to access the wide breath of agricultural experience from farmers.

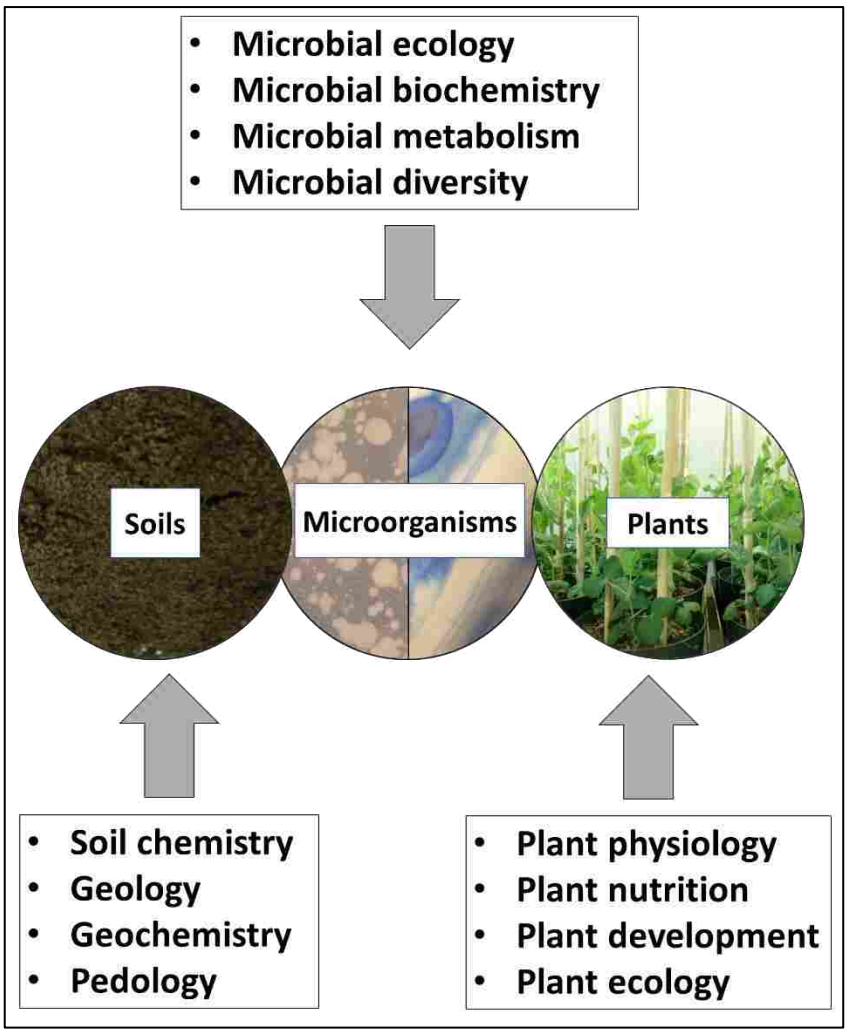


Figure 5.2: A few examples of the disciplines involved in researching the effects of carbonatite rock. Even in a relatively simple three-component system, the interconnected nature of the components means that a variety of tools, techniques, and perspectives is required to gain a full understanding of how the system responds to changes (i.e., the addition of carbonatite rock).

Literature cited:

Aguilar, A.S., and van Diest, A. 1981. Rock-phosphate mobilization induced by the alkaline uptake pattern of legumes utilizing symbiotically fixed nitrogen. Plant Soil **61**(1–2): 27–42. doi:10.1007/BF02277360.

Allen, T.F.H., and Starr, T.B. 2017. Hierarchy - perspectives for ecological complexity, 2nd Edition. University of Chicago Press, Chicago.

Arcand, M.M., Lynch, D.H., Voroney, R.P., and van Straaten, P. 2010. Residues from a buckwheat (*Fagopyrum esculentum*) green manure crop grown with phosphate rock influence bioavailability of soil phosphorus. Can. J. Soil Sci. **90**(2): 257–266. doi:10.4141/cjss09023.

Banfield, J., Barker, W., Welch, S., and Taunton, A. 1999. Biological impact on mineral dissolution : Application of the lichen model to understanding mineral weathering in the rhizosphere. Proc. Natl. Acad. Sci. **96**: 3404–3411. doi:10.1073/pnas.96.7.3404.

Bennett, P.C., Rogers, J.R., Choi, W.J., and Hiebert, F.K. 2001. Silicates, silicate weathering, and microbial ecology. Geomicrobiol. J. **18**(1): 3–19. doi:10.1080/01490450151079734.

Biswas, D.R., and Narayanasamy, G. 2006. Rock phosphate enriched compost: An approach to improve low-grade Indian rock phosphate. Bioresour. Technol. **97**(18): 2243–2251. doi:10.1016/j.biortech.2006.02.004.

Bland, W.L., and Bell, M.M. 2007. A holon approach to agroecology. Int. J. Agric. Sustain. **5**(4): 280–294. doi:10.1080/14735903.2007.9684828.

Burghelea, C., Zaharescu, D.G., Dontsova, K., Maier, R., Huxman, T., and Chorover, J. 2015. Mineral nutrient mobilization by plants from rock : influence of rock type and arbuscular mycorrhiza. Biogeochemistry **124**: 187–203. doi:10.1007/s10533-015-0092-5.

Calvaruso, C., Turpault, M.-P., and Frey-Klett, P. 2006. Root-associated bacteria contribute to mineral weathering and to mineral nutrition in trees: a budgeting analysis. Appl. Environ. Microbiol. **72**(2): 1258–1266. doi:10.1128/AEM.72.2.1258-1266.2006.

Carbonnel, S., and Gutjahr, C. 2014. Control of arbuscular mycorrhiza development by nutrient signals. Front. Plant Sci. **5**: 1–5. doi:10.3389/fpls.2014.00462.

Christie, R.V. 2019. Going back to the soil: An integrated approach to farming. Masters thesis, Wilfrid Laurier University. Available from https://scholars.wlu.ca/etd/2161.

Conway, G.R. 1987. The properties of agroecosystems. Agric. Syst. **24**(2): 95–117. doi:10.1016/0308-521X(87)90056-4.

Demoling, F., Figueroa, D., and Bååth, E. 2007. Comparison of factors limiting bacterial growth in different soils. Soil Biol. Biochem. **39**(10): 2485–2495. doi:10.1016/j.soilbio.2007.05.002.

Fierer, N., Jackson, J.A., Vilgalys, R., and Jackson, R.B. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. **71**(7): 4117–4120. doi:10.1128/AEM.71.7.4117-4120.2005.

Friese, C.F., and Allen, M.F. 1991. The spread of VA mycorrhizal fungal hyphae in the soil: inoculum types and external hyphal architecture. Mycologia **83**(4): 409. doi:10.2307/3760351.

Fuentes, J.P., Bezdicek, D.F., Flury, M., Albrecht, S., and Smith, J.L. 2006. Microbial activity affected by lime in a long-term no-till soil. Soil Tillage Res. **88**: 123–131. doi:10.1016/j.still.2005.05.001.

Ghiorse, W.C. 1988. The biology of manganese transforming microorganisms in soil. *In* Manganese in Soils and Plants. Springer Netherlands, Dordrecht. pp. 75–85. doi:10.1007/978-94-009-2817-6_6.

Gleissman, S.R. 2004. Chapter 2: Agroecology and agroecosystems. *In* Agroecosystem analysis. *Edited by* D. Rickerl and F. Charles. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, WI. pp. 19–29.

Grayston, S.J., Wang, S., Campbell, C.D., and Edwards, A.C. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol. Biochem. **30**(3): 369–378. doi:10.1016/S0038-0717(97)00124-7.

Hagedorn, C., and Holt, J.G.J.G. 1975. A nutritional and taxonomic survey of Arthrobacter soil isolates. Can. J. Microbiol. **21**(3): 353–361. doi:10.1139/m75-050.

Haynes, R., and Naidu, R. 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. Nutr. Cycl. Agroecosystems **51**(2): 123–137. doi:10.1023/A:1009738307837.

Helal, H.M., and Sauerbeck, D. 1986. Effect of plant roots on carbon metabolism of soil microbial biomass. Zeitschrift für Pflanzenernährung und Bodenkd. **149**(2): 181–188. doi:10.1002/jpln.19861490205.

Huang, P.M., Wang, M.-K., and Chiu, C.-Y. 2005. Soil mineral-organic matter-microbe interactions: Impacts on biogeochemical processes and biodiversity in soils. Pedobiologia **49**: 609-635. DOI: 10.1016/j.pedobi.2005.06.006.

Hungate, B., Danin, A., Pellerin, N.B., Stemmler, J., Kjellander, P., Adams, J.B., and Staley, J.T. 1987. Characterization of manganese-oxidizing (MnII→MnIV) bacteria from Negev Desert rock varnish: implications in desert varnish formation. Can. J. Microbiol. **33**(10): 939–943. doi:10.1139/m87-165.

Jones, J.M. 2016. Spanish River Carbonatite: Its benefits and potential use as a soil supplement in agriculture. Wilfrid Laurier University. Available from https://scholars.wlu.ca/etd/1795.

Keiluweit, M., Nico, P., Harmon, M.E., Mao, J., Pett-Ridge, J., and Kleber, M. 2015. Long-term litter decomposition controlled by manganese redox cycling. PNAS **112**: E5253-E5260. DOI: 10.1073/pnas.1508945112

Kernaghan, G. 2005. Mycorrhizal diversity: Cause and effect? Pedobiologia (Jena). **49**(6): 511–520. doi:10.1016/j.pedobi.2005.05.007.

Kiers, E.T., Duhamel, M., Beesetty, Y., Mensah, J.A., Franken, O., Verbruggen, E., Fellbaum, C.R., Kowalchuk, G.A., Hart, M.M., Bago, A., Palmer, T.M., West, S.A., Vandenkoornhuyse, P., Jansa, J., and Bucking, H. 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science (80-.). **333**(6044): 880–882. doi:10.1126/science.1208473.

Kirk, G.J.D., and Nye, P.H. 1986. A simple model for predicting the rates of dissolution of sparingly soluble calcium phosphates in soil. II. Applications of the model. J. Soil Sci. **37**(4): 541–554. doi:10.1111/j.1365-2389.1986.tb00386.x.

Lodwig, E.M., Hosie, A.H.F., Bourdès, A., Findlay, K., Allaway, D., Karunakaran, R., Downie, J.A., and Poole, P.S. 2003. Amino-acid cycling drives nitrogen fixation in the legume–*Rhizobium* symbiosis. Nature **422**(6933): 722–726. doi:10.1038/nature01527.

Mkhabela, M.S., Madani, A., Gordon, R., Burton, D., Cudmore, D., Elmi, A., and Hart, W. 2008. Gaseous and leaching nitrogen losses from no-tillage and conventional tillage systems following surface application of cattle manure. Soil Tillage Res. **98**(2): 187–199.

doi:10.1016/j.still.2007.12.005.

Myrvang, M.B., Hillersøy, M.H., Heim, M., Bleken, M.A., and Gjengedal, E. 2016. Uptake of macro nutrients, barium, and strontium by vegetation from mineral soils on carbonatite and pyroxenite bedrock at the Lillebukt Alkaline Complex on Stjernøy, Northern Norway. J. Plant Nutr. Soil Sci. **179**(6): 705–716. doi:10.1002/jpln.201600328.

Paustian, K., Andrén, O., Janzen, H.H., Lal, R., Smith, P., Tian, G., Tiessen, H., Noordwijk, M., and Woomer, P.L. 1997. Agricultural soils as a sink to mitigate CO_2 emissions. Soil Use Manag. **13**: 230–244. doi:10.1111/j.1475-2743.1997.tb00594.x.

Paustian, K., Six, J., Elliott, E.T., and Hunt, H.W. 2000. Management options for reducing CO_2 emissions from agricultural soils. Biogeochemistry **48**(1): 147–163. doi:10.1023/A:1006271331703.

Philippot, L., Raaijmakers, J.M., Lemanceau, P., and van der Putten, W.H. 2013. Going back to the roots : the microbial ecology of the rhizosphere. Nat. Rev. Microbiol. Microbiol. **11**(11): 789–799. doi:10.1038/nrmicro3109.

Rogers, J.R., and Bennett, P.C. 2004. Mineral stimulation of subsurface microorganisms: release of limiting nutrients from silicates. Chem. Geol. **203**(1–2): 91–108. doi:10.1016/j.chemgeo.2003.09.001.

Sage, R.P. 1987. Spanish River Carbonatite Complex. Ontario Ministry of Northern Development and Mines, Toronto, CA.

Shindo, H., and Huang, P.M. 1982. Role of Mn (IV) oxide in abiotic formation of humic substances in the environment. Nature **298**: 363–365. DOI: 10.1038/298363a0.

Smith, S.E., Dickson, S., and Smith, F.A. 2001. Nutrient transfer in arbuscular mycorrhizas: how are fungal and plant processes integrated? Funct. Plant Biol. **28**(7): 685. doi:10.1071/PP01033.

Uroz, S., Calvaruso, C., Turpault, M., and Frey-Klett, P. 2009. Mineral weathering by bacteria: ecology, actors and mechanisms. Trends Microbiol. **17**(8): 378–387. doi:10.1016/j.tim.2009.05.004.

Uroz, S., Calvaruso, C., Turpault, M.P., Pierrat, J.C., Mustin, C., and Frey-Klett, P. 2007. Effect of the mycorrhizosphere on the genotypic and metabolic diversity of the bacterial communities involved in mineral weathering in a forest soil. Appl. Environ. Microbiol. **73**(9): 3019–3027. doi:10.1128/AEM.00121-07.

VanVolkenburg, H. 2019. Exploring diversified vineyard ecological soil management strategies: Impacts of cover cropping, Spanish River Carbonatite, and smooth pigweed (*Amaranthus hybridus*) interactions on an agroecosystem. Masters thesis, Brock University.

Vestin, J.L.K., Söderberg, U., Bylund, D., Nambu, K., van Hees, P.A.W., Haslinger, E., Ottner, F., and Lundström, U.S. 2013. The influence of alkaline and non-alkaline parent material on Norway spruce tree chemical composition and growth rate. Plant Soil **370**(1–2): 103–113. doi:10.1007/s11104-013-1615-2.

Wen, Z., Li, H.H., Shen, Q., Tang, X., Xiong, C., Li, H.H., Pang, J., Ryan, M.H., Lambers, H., and Shen, J. 2019. Tradeoffs among root morphology, exudation and mycorrhizal symbioses for phosphorus-acquisition strategies of 16 crop species. New Phytol.: nph.15833. doi:10.1111/nph.15833.

Whitman, T., Neurath, R., Perera, A., Chu-Jacoby, I., Ning, D., Zhou, J., Nico, P., Pett-ridge, J., and Firestone, M. 2018. Microbial community assembly differs across minerals in a rhizosphere microcosm. Environ. Microbiol. **20**: 4444–4460. doi:10.1111/1462-2920.14366.

Zhang, L., Xu, M., Liu, Y., Zhang, F., Hodge, A., and Feng, G. 2016. Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium. New Phytol. **210**(3): 1022–1032. doi:10.1111/nph.13838.

Zhou, X., Liu, J., and Wu, F. 2017. Soil microbial communities in cucumber monoculture and rotation systems and their feedback effects on cucumber seedling growth. Plant Soil **415**(1–2): 507–520. Plant and Soil. doi:10.1007/s11104-017-3181-5.