THESIS

POTATO YIELD AND NUTRIENT ACQUISITION ARE SUPPORTED BY THE SOIL "BACTERIOME"

Submitted by

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ABSTRACT

POTATO YIELD AND NUTRIENT ACQUISITION ARE SUPPORTED BY THE SOIL "BACTERIOME"

Potatoes are the fourth largest food crop in the world; they are a staple food for much of South America and are the most consumed vegetable per capita in the United States. Breeding programs across the country seek to produce cultivars that are high yielding, disease resistant, and nutritious. The plant-soil-microbial community is greatly intertwined, each piece affecting the others. Soil microbial communities are highly influenced by edaphic features, and within a site microbial communities are influenced by the specific potato clone.

The first section of this project (Chapter 2) illustrates the variability in the bacterial root-associated community due to site and clone. The underlying core bacterial community of combined potato roots/rhizosphere soil that might benefit the quality of the potato crop was also examined. Root/rhizosphere soils from 18 different clones along with bulk soil bacterial communities from three sites (CA, CO, TX) were examined using 454 sequencing. In order to explain the soil bacterial potential, SPLS regression techniques were used to identify root-associated microbes correlated with tuber yield. Twenty-two bacterial operational taxonomic units (OTUs) were found to have a significant positive relationship with potato yield, many of these belonging to the bacterial order Rhizobiales. Interestingly, many of the bacteria identified in the SPLS regression have been studied in agricultural systems, however rarely in relation to potato. Further study of the relationship between potato plants and these microbes is warranted.

Parts of South America, where potato is a staple food, have been described as good candidates for the implementation of biofortified foods; additionally, the potato is a good candidate for biofortification. Biofortification of foods through plant breeding can increase

essential nutrients in staple crops to decrease human disease and mortality. The second aspect of this project (Chapter 3) assessed the impact that soil nutrient contents, soil bacterial diversity, and potato clones have on tuber nutrient contents. A predictive model was created to address the degree to which these independent predictors impact the tuber nutrient levels of N, P, K, Zn, Fe, Mn and Cu. Soil nutrient levels and bulk soil bacterial diversity had a similar ability to increase tuber nutrient levels. Increasing soil bacterial diversity was shown to support acquisition of these seven nutrients. This indicates that management practices to increase soil bacterial diversity may support plant nutrient acquisition, thus lowering fertilizer use.

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CHAPTER 1

INTRODUCTION

With an estimated 10 billion bacteria in a single gram of soil, scientists have a virtually endless supply of organisms to study from a handful of soil (Whitman et al., 1998). Humanity benefits from soil microorganisms in the form of antibiotics, treatment for iron poisoning and crop disease reduction by plant growth promoting bacteria (Waksman and Woodruff, 1940; Kloepper et al., 1980; Miller, 1989). Agriculture relies on healthy soils for crop success. Build-up of a pathogen in the soil can cause major damage to a food system, for example the plant pathogen *Phytophthora infestans* was the causal agent of the Irish potato famine (Schumann, 1991). Other soil disease states are linked to microbial disturbance; low fungal diversity improves invasive plant species success (Broz et al., 2007) and removal of rare bacterial species increases aphid size and feeding damage (Hol et al., 2010). However, disease suppressive soils exist where a pathogen is kept in check by other soil organisms (Haas and Défago, 2005).

Field site soil and crop cultivar each influence resident microbes to some degree (İnceoğlu et al., 2011; Peiffer et al., 2013a). Altering soil microbial communities is desirable to promote plant growth and suppress disease. Less concrete is how to best change soil microbial communities and the members who should comprise the new or changing microbial community.

Breeding programs currently endeavor to develop cultivars with high yield, disease resistance and health properties such as antioxidant activity (Stushnoff et al., 2008). Adopting management practices which include clones that are not only healthier for human consumption, but also support a healthy soil microbiome is advantageous for ecosystem stability. The Western Regional Potato Trials, a collaborative effort between several universities and the USDA-ARS, seeks to improve the potato germplasm. This program has tested potato clones for field growth

characteristics, disease resistance and culinary quality since 1998 (Novy and Stark, 2012). Each site follows the best production practices as determined by the growers and field conditions. Using potato clone samples from these trials enabled the study of both site and clone effects on the soil microbial community.

As potatoes are the world's fourth most grown crop and a staple food for many cultures, research supporting their growth could be beneficial to many people. Potatoes have high nutritional value for protein and mineral content and use less water to provide more dry matter and nutrients than cereal grains (Bamberg and Del Rio, 2005; Burgos et al., 2007). Due to high variability in potato tuber iron content, it has been considered a good candidate for biofortification of essential minerals (Haynes et al., 2012).

The aims of this project are:

1) Identify the degree to which site and clone impact potato root-associated soil bacteria communities, and to characterize a core set of bacteria that are beneficial for crop yield.

2) Identify the degree to which site and cultivar impact the mineral contents of potato tubers to understand the value of microbial communities in supporting mineral acquisition.

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CHAPTER 2

SITE AND CULTIVAR EFFECTS ON THE POTATO ROOT-ASSOCIATED CORE MICROBIOME AND ITS RELATIONSHIP TO TUBER YIELD

Chapter Summary

Potatoes are the fourth most grown food crop in the world with consumption increasing worldwide, especially in developing nations. Potato breeding programs focus on favorable genetic characteristics which enhance disease resistance and culinary quality; in addition, some programs are beginning to focus on anti-cancer activity of potatoes. Researchers concentrating on the bacterial inhabitants of potato soil and roots report that communities are highly influenced by edaphic features, and within a site microbial communities are influenced by the specific potato clone. The aim of this study was to describe the variability in the root-associated bacterial community due to site versus clone, and to determine whether an underlying core bacterial community exists that might benefit the quality of the potato crop. Using 454 sequencing, root-associated microbial communities of 18 potato clones grown in three states (CA, CO, TX), as well as the bulk soil, were examined. Variance analysis using perMANOVA attributed 45.4% and 24.1% of the community variability to site and clone effects, respectively. Sparse partial least squares (SPLS) regression techniques were used to identify root-associated microbes correlated with tuber yield. A total of 22 bacterial operational taxonomic units (OTUs) were positively correlated with tuber yields; the majority of these belong to the order Rhizobiales with 5 of the top 13 predictors belonging to the genus *Devosia*. Interestingly, no known symbiotic relationship between potato roots and nitrogen fixing bacteria exists.

Introduction

The soil-plant-microbial system is complex with positive and negative interactions occurring between every component. Improving positive interactions for better crop yield is

desirable as this could improve overall soil health and reduce the need for chemical inputs. Understanding the various contributions of soil, plant genotype, and microbes on crop and soil quality can help scientists and managers better focus their efforts and resources to achieve the greatest benefits.

Microbial community composition is driven by multiple factors. For example, plant richness (i.e., the number of different plant species present) can influence the Actinomycete population, while edaphic features are correlated with the presence of specific bacteria (Bakker et al., 2013). Crops also shape soil microbial populations over time. For example, fluorescent Pseudomonad populations decrease throughout maize growth, while total microbial population numbers remain steady throughout the season (Chiarini et al., 1998). Plants also influence the rhizosphere, Betaproteobacterial communities shift throughout the growth cycle of different potato clones, becoming more similar towards plant senescence (İnceoğlu et al., 2010). Shifts in the microbial community can bring about changes in environmental stability. For example, success of an invasive plant species occurs in conjunction with decreased fungal abundance and diversity (Broz et al., 2007). In addition, the removal of rare microbial species, and hence diversity, can lead to an increase in aphid size and possible feeding damage (Hol et al., 2010). Understanding that these complex facets of the plant-soil-microbial community exist can help elucidate microbial plant growth promotion modes of action. For example, antagonistic effects against *Fusarium* wilt by *Streptomyces* sp. were lost when $FeCl_3$ was added to reaction plates, this shows that iron limitation is required for the success of *Streptomyces* sp. when *Fusasium* is encountered (Cao et al., 2005). However, altering the soil environment for crop health can be difficult. Specificity of bacterial strain, host genotype and growth conditions each play a role in success of plant growth promotion by microbial inoculation (Belimov et al., 2001). Imposing

and understanding changes in microbial populations to increase crop productivity is the next step towards more sustainable farming methods.

Potato is a globally relevant crop, as it is a staple food for many cultures and the most consumed vegetable in the United States. The average American consumes approximately 130 pounds of potato per year (Bohl and Johnson, 2010). The relationship of potato plants with soil bacteria has been, and continues to be, of great interest since bacteria can increase plant growth and plants can influence soil microbes through root exudation (Badri et al., 2009). Pseudomonads have been well studied with potato plants as they have specifically been linked to potato plant growth promotion (Kloepper et al., 1980; Conn et al., 1997). A rhizosphere and cultivar influence over soil bacteria exists; however, the largest determinant of soil microbial populations tends to be the geographic location where samples were collected (İnceoğlu et al., 2011). Clarifying the degree of influence soil bacteria, sampling location, and plant cultivar have on crop success will help farmers and researchers determine where to put resources for best overall crop improvement.

While it is well established that potato soil bacterial communities shift according to site and cultivar, describing a set of bacteria common to all sites and cultivars could uncover potato competent microbes important for crop productivity and health in a variety of locations. Defining a "core bacteriome" can be elusive, one example describes the core microbiome of humans as a "set of genes present in a given habitat in all or the vast majority of humans" (Turnbaugh et al., 2007). This definition is broad and does not take into account abundance of genes or taxonomy. The question of interest can also influence how a researcher defines a core microbiome. For example if persistence, abundance, or presence of specific bacteria are of interest, the data analysis, and hence, core microbiome definition will change (Shade and

Handelsman, 2012). The objective of defining a "core bacteriome" in this project was to identify bacterial candidates that are likely to improve potato crop health.

The overall aim of this study was to identify the extent to which different sites and potato selections/cultivars influence root-associated bacteria; then, using the known bacterial community from each cultivar and site, define a core community that is common among potato sites and/or clones. Root-associated bacterial populations from 18 potato clones from three of the Western Regional Trial (Novy and Stark, 2012) locations (CA, CO, TX) were assessed by pyrosequencing. Community variability (i.e., site and clonal effects) and stability (i.e., core microbiome) were analyzed based on taxonomic abundances. In addition, the potential relationship between tuber yields and bacterial average abundances were analyzed by sparse partial least squares (SPLS) analysis.

Materials and Methods

Plant root and rhizosphere soil samples were collected at harvest just prior to vine kill from each clone (n = 3) from the Western Regional Potato Trials in 2011 and stored at negative 20°C until extracted. Samples were collected from each of three sites (Tulelake, California; San Luis Valley, Colorado; and Springlake, Texas), shipped fresh, and moved to cold storage (-20 °C). At least 15 bulk soil samples (5 to 10 g) were randomly taken from the top approximately three to five inches of soil throughout the potato field at each site at the same time as plant collection. Random bulk soil samples were pooled, homogenized in a 2 mm sieve, and stored at -20 °C. Approximately five inches of roots along with the adhered rhizosphere soil were taken from each plant. Roots and rhizosphere soil (0.5 g per sample) were frozen with liquid nitrogen and pulverized in an IKA A11 basic grinder (Wilmington, North Carolina). Roots and rhizosphere soil from the three replicate plants collected at each site were pooled and one DNA

extraction per clone root/rhizosphere per site was performed where sufficient sample was available. Microbial DNA was extracted using MoBio PowerSoil-htp kit (catalog # 12955-4, Carlsbad, California) and stored at -20 °C until further processing, this bacterial DNA is hereafter called the root-associated community. In addition, 0.5 g bulk soil was extracted in duplicate following the same procedure as root-associated DNA.

A quantitative PCR (qPCR) for the bacterial 16S rRNA genes was performed with the following primers: 27F, 5#-cctccctcgcgccatcagnnnnnnnnnnnnAGAGTTTGATCMTGGCTCAG-3# and 388R, 5#-gccttgccagcccgctcagTCTGCTGCCTCCCGTAGGAGT-3# (Badri et al., 2009). The lowercase sequence of the primers are necessary adapters for binding and amplification used in pyrosequencing, the uppercase regions are primers targeted to conserved regions of the 16S rRNA gene (Lane et al., 1985; Lane, 1991; Marchesi et al., 1998). The "n" repeat represents the position of unique barcodes (Hamady et al., 2008) that allow identification of each individual sequence read with its original PCR reaction (i.e., site/clone). Amplification was performed using Thermo Scientific Maxima SYBR (cat #K0241, Waltham, Massachusetts) master mix and thermal cycling program as follows: 1) 95 ºC for 8.5 min, 2) 95 ºC x 15 sec, 55 ºC x 15 sec, 72 ºC x 30 sec, repeated 35 times, 3) 72 ºC x 5 min. Samples were run through Lonza FlashGel DNA Recovery System (Basel, Switzerland) to purify amplified DNA from other fragments. Each sample was then quantified using KAPA Biosystems qPCR kit following the manufacturer's instructions (Cat #kk4802 for GS Titanium DNA standards and primer premix kit and Cat #kk4607 for SYBRFAST Bio-Rad i-Cycler qPCR kit). Amplified DNA samples were pooled in equimolar ratios and re-quantified using the KAPA Biosystems qPCR kit. Once the library was quantified, it was diluted and used at a concentration of four copies per bead with the Roche GS Junior Sequencing System. Sequencing was performed according to the Roche GS

Junior protocol (Branford, Connecticut). Samples that were sequenced are listed in Appendix, Table A.2.1.

Statistical Analysis

Bacterial library read editing and processing was performed with Mothur Ver 1.24 (www.mothur.org) (Schloss et al., 2009). Sequences were then trimmed based on a minimum of 360 and a maximum of 720 flows (total flows = 800). Sequencing errors were removed by shhh.flows, which is Mothur's implementation of Amplicon Noise (Quince et al., 2009). Primers and barcodes were removed allowing for two errors in primer sequence and one error in barcode sequence, eight maximum homopolymers were allowed per sequence. Unique sequences were then extracted to reduce redundancy in the dataset for faster processing time. Sequences were aligned to the SILVA 16S rRNA bacteria alignment. All sequences were aligned to the same genetic space (SILVA 16S alignment positions 1044 - 6426). Gaps in sequences were removed (vertical=T and trump=.). Sequences were further de-replicated. Pre-clustering of sequences was performed and chimeras were identified, and then removed with chimera.uchime. Sequences were classified to the Mothur RDP traningset 9 to identify and remove non-bacterial sequences. Distances were calculated using dist.seqs (cutoff=0.15), and clustered (method=furthest, cutoff=0.10). The furthest method was chosen since it most closely represents the current recommendations for taxonomic classification and identification of new species (Stackebrandt, 2006) Alpha and beta diversity measurements for 1000 sequences per sample were selected using the subsample command in Mothur which reports the average value for 1000 iterations. A custom database was made by combining all of the type strain sequences found in the SILVA SSU Ref NR database Version 108 (http://www.arb-silva.de) database (10,509 type sequences) and selected sequences from the RDP training set Vers. 9

(http://www.rdp.cme.msu.edu) that were under-represented in the SILVA database. RDP phyla that are included in the custom database (and their represented number) are: Acidobacteria (405), Armatimonadetes (107), BRCI (12), ODI (33), OP11 (18), SR1 (10), TM7 (15) and W53 (13). Quality sequences were then matched to the custom database to identify the taxonomy of observed OTUs.

Distance measurements previously calculated were then used for PCoA and AMOVA analysis using the Odum distance measurement (Odum, 1950). The OTUs identified by name were used in subsequent figures and tables. PerMANOVA analysis was performed in the R vegan package (Oksanen et al., 2013). Sparse partial least squares analysis was performed in the R SPLS package (Chun and Keles, 2010).

Results

Diversity and Evenness

The bulk soil (Figure 2.1) and root-associated (Figure 2.2) bacterial communities consisted of 16 different phyla. Visually, Gammaproteobacteria are enriched in root-associated bacterial samples (Figure 2.2) and Acidobacteria are enriched in bulk soil bacterial samples (Figure 2.1). Richness, which is a measure of the number of species or taxonomic units of interest, varied among sites for both bulk soil ($p < 0.001$) and root-associated ($p < 0.001$) bacteria based on an operational taxonomic unit (OTU) with a genetic distance of 3% (Table 2.1). Texas had the highest bulk soil and root-associated OTU richness of the three sites (Table 2.1). Bulk soil OTU richness was 682, 521, and 248 for the TX, CO, and CA sites, respectively; and root-associated OTU richness was 387, 294, and 291 for the TX, CO, and CA sites, respectively. Root associated and bulk soil bacterial DNA diversity was determined by Shannon's entropy (H) and Simpson's index (D). Simpson's index for both bulk and root-associated bacteria was different

among sites ($p < 0.001$ and $p = 0.015$, respectively, Table 2.1). Shannon and Simpson diversity indices agree, with the TX site having the highest diversity for both bulk and root-associated bacteria (Table 2.1). Bulk soil samples were not statistically different from each other for either measure of evenness ($p = 0.221$); however, root-associated evenness was significantly greater at the TX site ($p < 0.001$), as compared to either CO or CA (Table 2.1). Principle coordinates analysis (PCoA) reveals that root-associated bacterial communities separate primarily by site (Figure 2.3); however, variance analysis by perMANOVA ascribed 45.4% variability to site and 24.1% to plant variation within site.

"Core Bacteriome"

The average abundances of OTUs within each sample were used to describe a set of "core bacteriomes" or bacterial OTUs that were (1) present at all three sites and (2) present in a specified percentage of the samples at each site. For example, from the combined pool of 15 random bulk soil cores, two bulk soil samples per site were sequenced, thus the definition of a core was described on two levels: designated as presence of OTU in at least one sample (50% of samples) per site or presence of OTU in both samples (100% of samples) at each site. Under the less stringent definition, the "core" bacteriome in the bulk soil samples consisted of just 95 OTUs or 2.28% of the OTUs present; however, they comprise nearly 25% of the total bacterial community (abundance) (Figure 2.4). When the "core" definition was made more stringent, both the number (nine) and abundance (0.2%) of OTUs identified as belonging to the core bacteriome declined dramatically (Figure 2.4). A list of the nine bacterial OTUs present in 100% of the bulk soil samples and the average abundance of each OTU at all three sites are provided in Table 2.2.

At least 11 root-associated bacterial DNA samples were sequenced at each site (Appendix Table A.2.1), allowing for "core bacteriome" definitions for presence at all three sites at 10%, 25%, 50%, 75%, 90% and 100%. At least 75% of samples contained 8 OTUs or just 0.07% of the OTUs present, this small number of OTUs comprises approximately 15% of the total individuals sequenced (Figure 2.5). Using a less stringent definition, 74 OTUs (0.61% of OTUs present) are in at least 25% of samples; however these 74 OTUs made up about 35% of the total community which is over double the total community members in the "core bacteriome" from the previous definition (Figure 2.5). Six of the total eight OTUs present in 75% of root/rhizosphere samples are in the Rhizobiales order, four of those six are in the genus *Devosia* (Table 3). In 50% of all root-associated bacterial samples, 24 OTUs are present, 12 of these belong to Rhizobiales and five are in *Devosia* (Table 2.4).

Correlating Root-Associated Bacteria to Yield

Total yield for each clone involved in the Western Regional Potato Trials is measured yearly (Novy and Stark, 2012). Sparse partial least squares (SPLS) analysis (R package SPLS) was used to determine the relationship (i.e., loadings) between individual OTU abundances and yield (Chun and Keles, 2010). A loading is the scale relationship a particular OTU has with yield and is reported if found to be significant, either positive or negative. The SPLS package performs 1000 bootstraps to help eliminate false positives, allowing for better confidence in results over other types of correlation analyses. All five members of the genus *Devosia* have positive loadings with yield; four of the total eight bacteria in the presence/absence core definition are also *Devosia*. Two members of the genus *Pseudoxanthamonas* appear in the "core

bacteriome" SPLS analysis, one with a negative loading, the other positive; presence/absence definitions for core did not produce any *Pseudoxanthamonas* spp.

Discussion

The first aim of this study was to identify the extent of soil and cultivar impact on potato root-associated bacteria. A visual inspection of the PCoA analysis shows separation largely by site followed by clone. Further analysis by perMANOVA illustrates differences in bacterial community were foremost driven by site, 45.4%, then by plant variation within site, 24.1%. Due to the cost of pyrosequencing, plant replicates were not run at each site thus, specific shifts in bacterial communities due to clone cannot be examined with this dataset. Some of the variation seen in this dataset, however, is likely due to clonal selection as Manter et al. (2010) showed that bacterial communities from the same potato clone cluster together in canonical correspondence analysis. Similar to our findings, plant to plant variation in the maize rhizosphere microbiota was estimated to be 19.1% (Peiffer et al., 2013b). Shannon and Simpson diversity indices were significantly different between the three sites for both bulk soil and root-associated bacteria. Diversity of root-associated bacteria was lower than bulk soil bacteria at every site implying a more selective growth environment within the plant root zone. A number of studies have shown the heavy influence edaphic factors exhibit over soil microbial communities (Chiarini et al., 1998; Bossio et al., 1998; Fierer et al., 2007; İnceoğlu et al., 2010). While edaphic features primarily shape bacterial populations in the soil, plants also influence the soil microbial community surrounding their roots (Chiarini et al., 1998; İnceoğlu et al., 2011; Knief et al., 2011). Cultivars may have a smaller influence on the components of the microbial community; however, cultivar-specific differences may be important as they may be correlated with plant performance, such as plant biomass (Manter et al., 2010).

A core bacterial community that is common to potato bulk soil and root-associated bacteria was then examined to determine candidates that may support potato plant growth at all locations regardless of clone. Describing beneficial microbial residents is not uncommon. Endophytic bacteria of multiple clones has been described through pyrosequence analysis on one site (Colorado), however this dataset cannot account for the site variation that profoundly affects the resident microbial population (Manter et al., 2010) . Other studies have investigated smaller portions of the soil microbiome such as observing changes within Betaproteobacteria, one class of the Proteobacteria phylum (İnceoğlu et al., 2011). Focusing on culture techniques to identify plant growth promoting bacteria does not necessarily limit observations to specific taxa, however the community is then restricted to only those bacteria which are culturable (Sessitsch et al., 2004; Andreote et al., 2009; Diallo et al., 2011).

Of the nine OTUs present in 100% of the bulk soil samples, three (30%) belong to the order Rhizobiales, whereas in the eight OTUs present in 75% of all clones in root-associated bacteria, six (75%) belong to the order Rhizobiales. Members of the genus *Devosia* represent half of the eight total root-associated core bacteria that exist in at least 75% of all clones at all three sites. Another study found *Devosia* to be a major contributor to potato endophyte populations in clones in Colorado (Manter et al., 2010). *Devosia* is a nitrogen-fixing bacteria and has been shown to grow in diesel-contaminated soil (Rivas et al., 2002; Vanparys et al., 2005; Ryu et al., 2008). The presence and possible significance of the *Devosia* species in these Western Regional Potato Trial results warrants further study. Two genera of the Rhizobiales order, *Bradyrhizobium* and *Rhizobium*, are also part of the root-associated bacteriome of 75% of the clones. Both genera form nitrogen fixing nodules with legume plants and are considered to provide a significant portion of nitrogen to these crops (Fred et al., 1932; Pagan et al., 1975;

Kosslak et al., 1987; Kaneko et al., 2002; Boone et al., 2005). Potato roots are not known to form nodules with *Rhizobium*, however lipopolysaccharide from *Rhizobium etli* induces systemic resistance to infection by a cyst nematode and *Rhizobium* species isolated from soils planted with alfalfa and potato increase growth of different potato cultivars (Sturz and Christie, 1998; Reitz et al., 2000).

Pseudomonas and *Sphingobium* genera are included in 75% of all root-associated bacterial clone samples. A 2011 review of the potato rhizosphere describes *Pseudomonas* and members of the Sphingomonadales order (which includes *Sphingobium)* as major members of the potato rhizosphere community (Diallo et al., 2011). *Pseudomonas* species have long been studied as helpful members of the potato root bacterial community, some strains are able to suppress the potato pathogen *Phytophthora infestans* (Andreote et al., 2009). These bacteria are members of the 75% "core bacteriome." This census of the potato root-associated core bacterial community describes foundational members of this ecosystem, but potential function of these community members cannot be determined from this analysis.

Core microbiomes have been described in a variety of other systems including *Arabidopsis thaliana*, maize and the human gut (Turnbaugh et al., 2007; Lundberg et al., 2012; Peiffer et al., 2013b). Maize rhizosphere is enriched for the bacterial orders Burkholderiales, Oceanospirillales and Sphingobacteriales, and the genus *Sphingobium* (Peiffer et al., 2013b). Two members of the Burkholderiales family were observed in at least 50% of all root-associated samples and one member of the genus *Sphingobium* occurred in at least 75% of potato clones in the present study. Within the endophytic compartment of *Arabidopsis thaliana,* core bacterial families are Rhizobiaceae, Methylobacteriaceae, Pseudomonadaceae and Moraxellaceae (Lundberg et al., 2012). Four members of the Rhizobiaceae family and two members of

Pseudomonadaceae family inhabited at least 50% or more of all root-associated bacterial samples sequenced here. A few similarities between the current study "core bacteriome" for potato and core bacterial communities of maize and *Arabidopsis thaliana* are seen, however it appears that each species' core bacterial community is quite unique.

There is no common way to define a "core microbiome" and it has been suggested that a definition should be based around project goals (Shade and Handelsman, 2012). With respect to this notion, defining a "core bacteriome" based on a desired trait or process may be more helpful than describing a core based on bacterial presence/absence, which is highly dependent upon the inclusion criteria of the core. Therefore, a bacterial core correlating to yield was also assembled using SPLS regression. There are two main benefits to this approach: (1) No *a priori* assumptions of thresholds are required and (2) the identified members are directly linked to a process, yield, in this case. SPLS regression uncovered a number of bacterial OTUs within the same genus that have vastly different correlations to yield; showing that assumptions of bacterial function based off general taxonomy alone are inaccurate. In fact, metaproteogenomic analysis of the phyllosphere and rhizosphere of rice revealed that protein families of different functional guilds varied among sites and the authors concluded that the microbiota make general adaptations on an individual basis rather than taxonomic specific adaptions to the phyllosphere (Knief et al., 2011).

Sparse partial least squares analysis suggests that some of the samples identified in the 75% core root-associated bacteriome have a positive effect on tuber yield. The genus *Devosia* is again present with five OTUs having a positive loading, one with the highest positive correlation to yield. One *Brevundimonas* species (*intermedia*) was highly positively correlated with tuber yield. *Brevundimonas* has been noted as a minor component of the bacterial population in the

potato rhizosphere (Diallo et al., 2011). Two other studies have linked *Brevundimonas* to potato root and shoot biomass (Manter et al., 2010) and plant growth promotion in potato along with siderophore production (Sessitsch et al., 2004). Common potato and root-associated bacterial genera are uncovered in SPLS this helps authenticate statistical analysis with previously culture microbes which have proven beneficial to potato plants and *in silico* methods. The *Asticcacaulis* genus along with *Pseudomonas fulva,* both have positive loadings to yield and both bacteria have been studied in soils with soybean roots as endophytes and epiphytes (Kuklinsky-Sobral et al., 2004). These soil bacteria are not classically associated with potatoes but, they may provide growth support to increase potato yield.

Identifying the core microbes that are likely to support crop health can help growers use soil biological management practices and fewer chemical inputs to support desirable crop traits. This concept has been demonstrated in a greenhouse study, by adding a plant growth promoting rhizobacterial and arbuscular mycorrhizal fungus mixture to tomatoes, fertilizer rates can be decreased by 25% without losing any integrity of the crop (Adesemoye et al., 2009). Using the present study as a springboard, investigators may isolate known helpful bacteria from the soil and apply cultures of these organisms to potato plants to biologically target a desired function of plant growth.

Figures and Tables

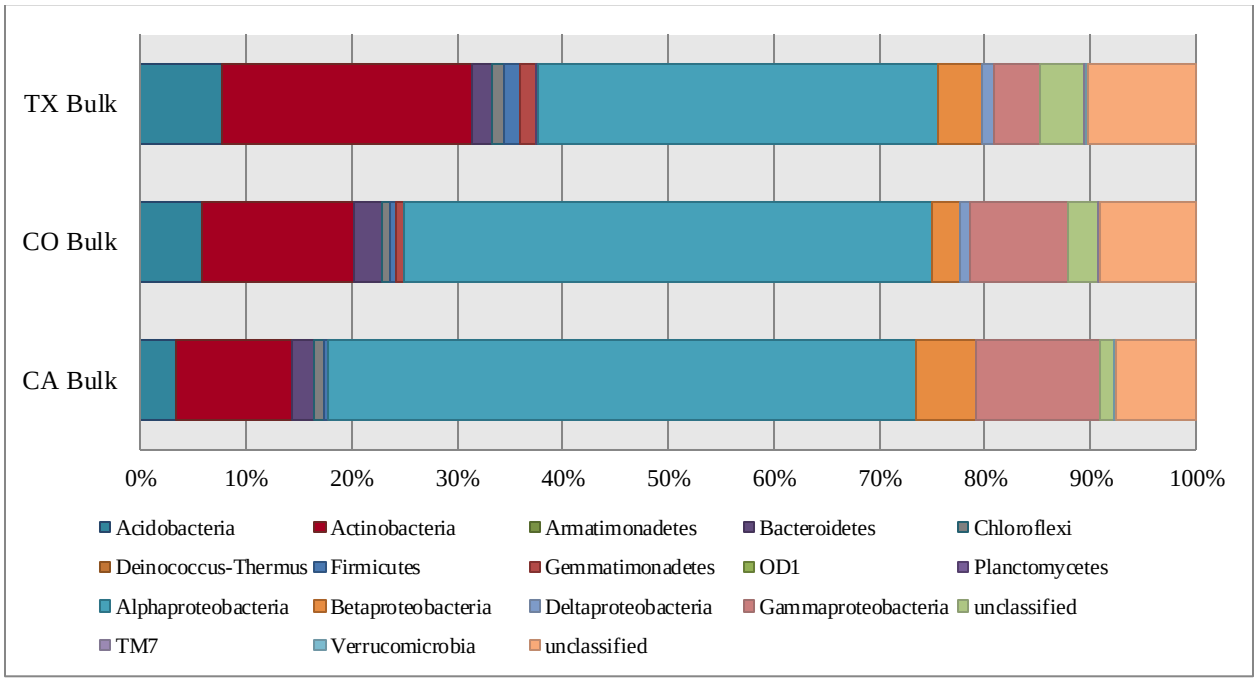

Figure 2.1. Phyla/Class relative abundances (%) in the bulk soil samples at three different sites of the Western Regional Trials.

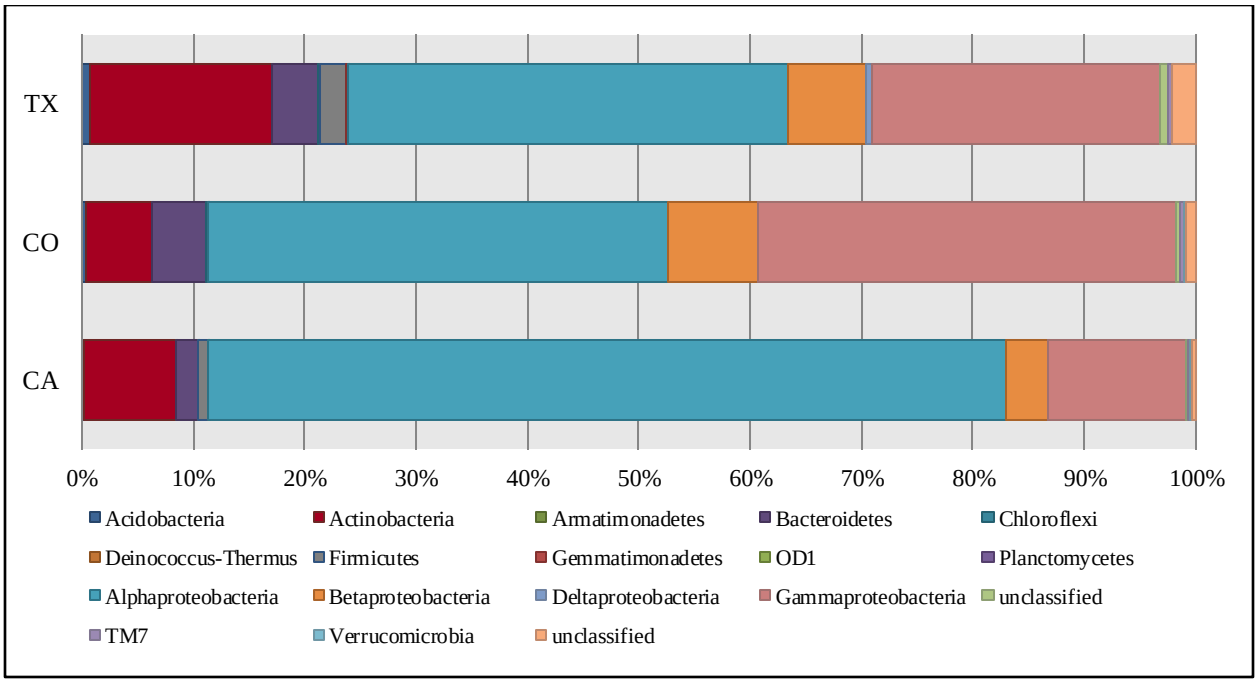

Figure 2.2. Phyla/Class relative abundances (%) in the root/rhizosphere soil samples at three different sites of the Western Regional Trials.

Table 2.1. Alpha diversity indices for bulk soil and root/rhizosphere soil samples at three sites of the Western Regional Trials. All values for 1000 reads and 3% genetic distance. *n:* number of samples sequenced, S_{obs}*:* OTU richness, *H:* Shannon's diversity index, exp(*H):*normalized Shannon's diversity index, or "true" diversity (Jost, 2006), E_H*:* Shannon's evenness, *1/D:* 1 / Simpson's diversity index, E_D*:* Simpson's evenness. *p*-values were determined by AMOVA.

Source	State	*n*	S_{obs}	*H*	exp(*H*)	E_H	1/*D*	E_D
Bulk soil	CA	2	248	4.623	102	0.842	52	0.232
	CO	2	521	5.708	301	0.914	256	0.439
	TX	2	682	6.285	537	0.963	559	0.814
	p-value		<0.001	0.046	0.072	0.221	0.015	0.278
Root/rhizosphere soil	CA	26	291	4.333	76	0.765	30	0.092
	CO	23	294	4.274	72	0.751	32	0.098
	TX	11	387	5.080	161	0.853	82	0.202
	p-value		<0.001	0.003	<0.001	0.009	<0.001	<0.001

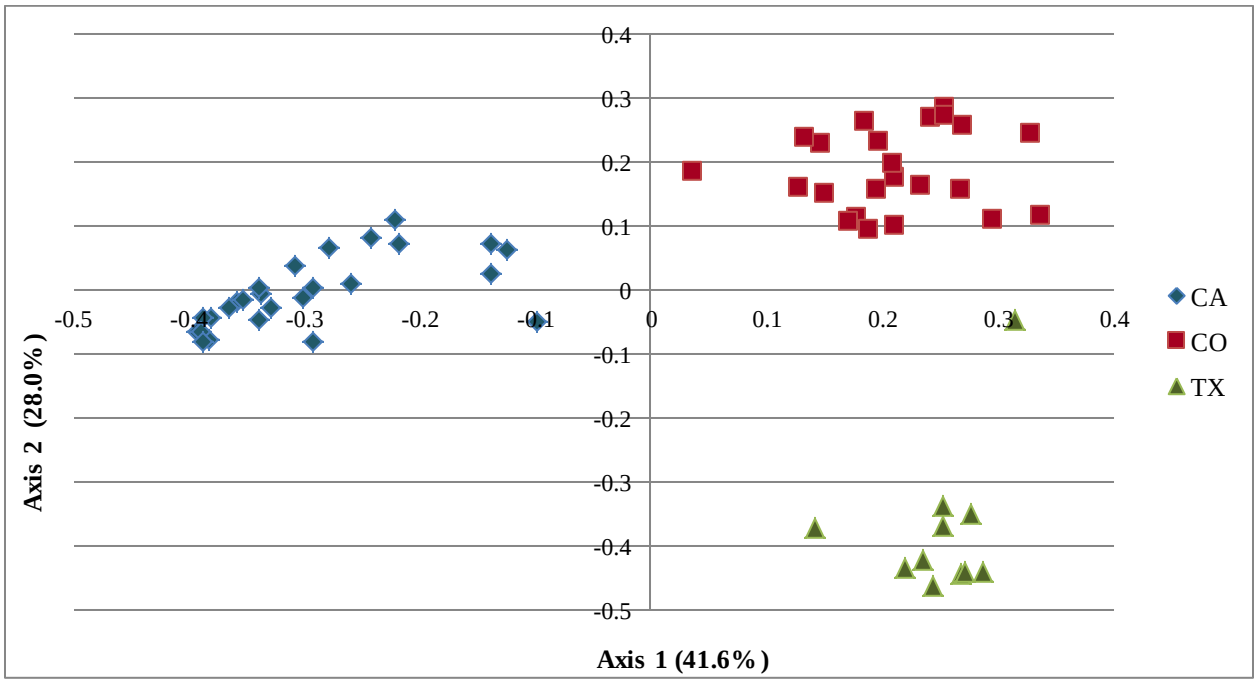

Figure 2.3. Principle Coordinate Analysis (PCoA) of the root/rhizosphere samples using Odum distances for three sites of the Western Regional trials.

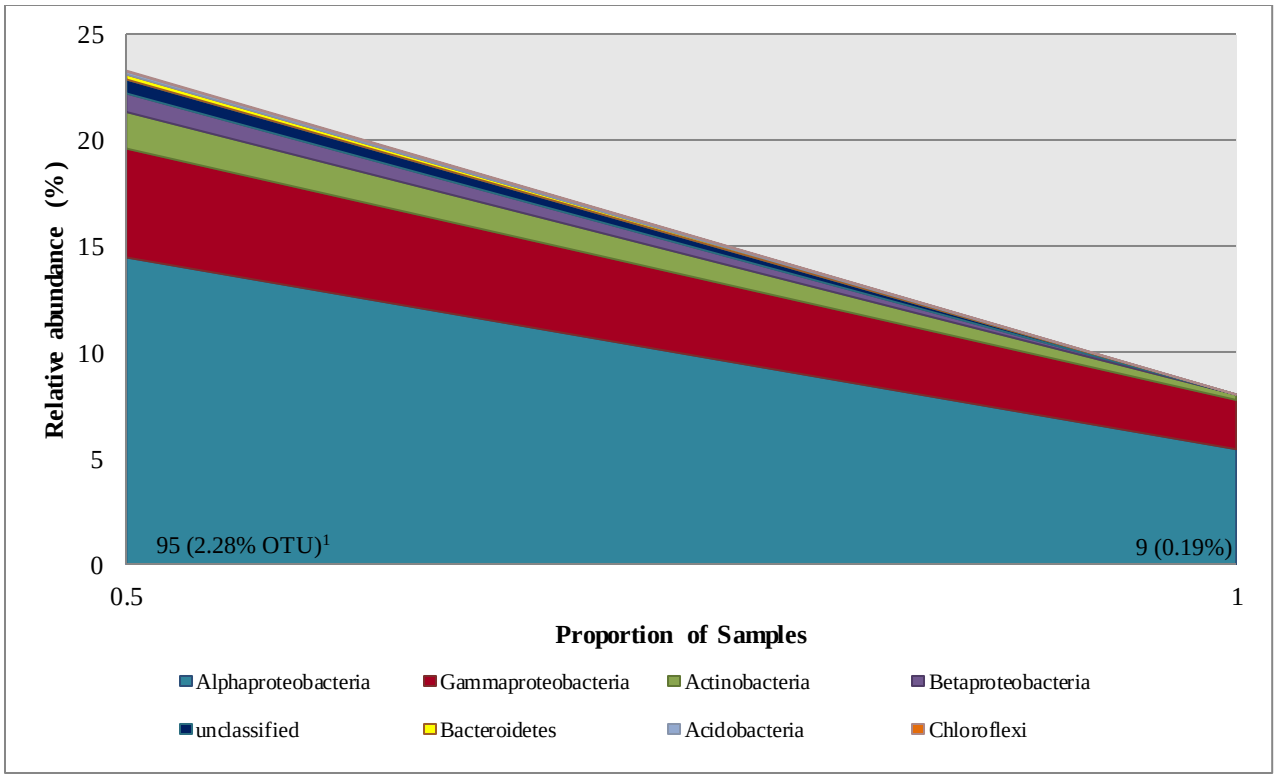

Figure 2.4. Composition of the core bacteriome identified in bulk soil samples at three sites of the Western Regional Trials using differing sample proportion thresholds. The y-axis is the relative abundance (%) of the individual identified as "core" and is color-coded by Phyla/Class (see legend). The x-axis is the proportion of samples in which the core OTU was present at all three sites. [1]Number (and percent of the total) of core OTUs identified.

Table 2.2. Bulk soil core bacteriome: OTUs present at all three sites and 100% of the samples from each site. Average abundance in samples sequenced of each OTU is listed per site.

					Average Abundance		
OTU	Class	Order	Family	Genus	CA	CO	TX
Otu001	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Brevundimonas*	3.41	0.57	0.28
Otu002	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	6.56	0.18	0.22
Otu042	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*Steroidobacter*	0.96	1.88	0.84
Otu069	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Rhizobium*	1.32	2.76	0.20
Otu072	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	*Microvirga*	0.30	1.23	0.16
Otu115	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*unclassified*	0.22	0.19	0.36
Otu208	Actinobacteria	Actinomycetales	Geodermatophilaceae	*Blastococcus*	0.53	0.17	0.19
Otu230	Actinobacteria	Actinomycetales	Nocardioidaceae	*Nocardioides*	0.17	0.40	0.05
Otu313	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Rhodanobacter*	0.69	0.13	0.13

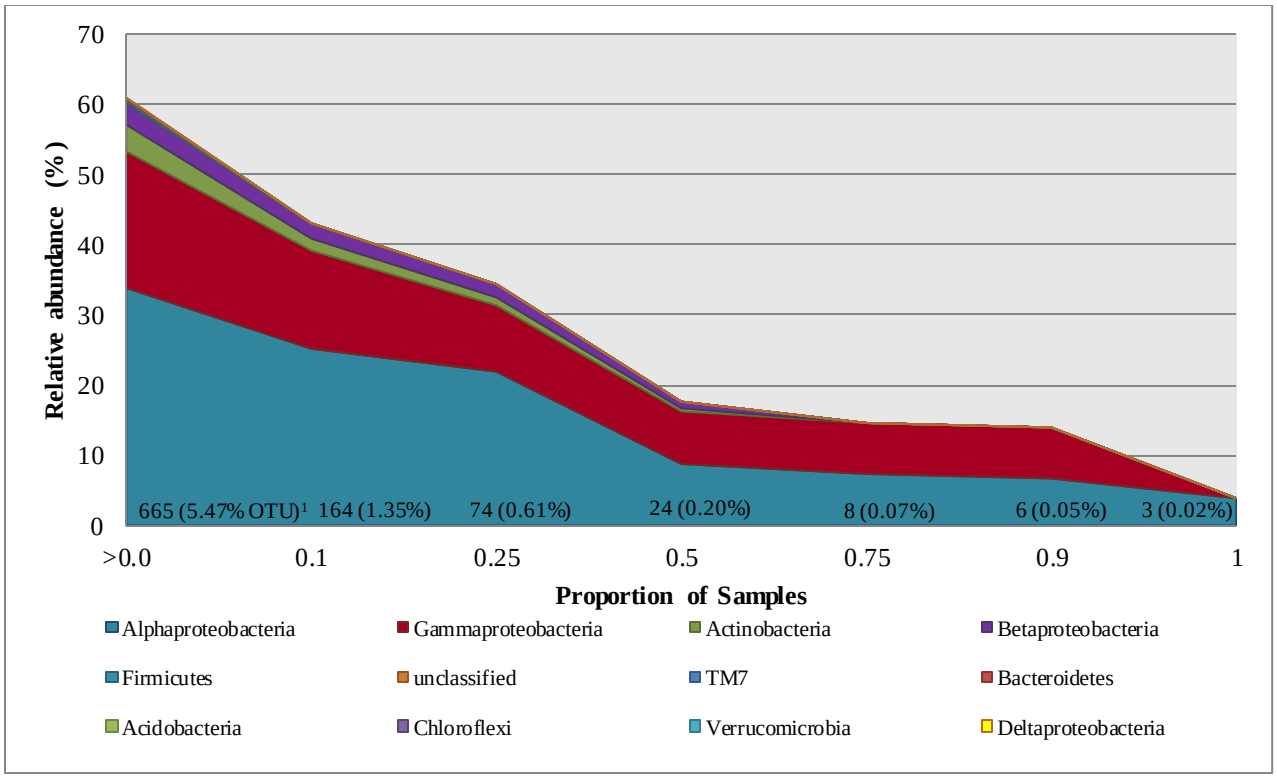

Figure 2.5. Composition of the core bacteriome identified in the root/rhizosphere soil samples at three sites of the Western Regional Trials using differing sample proportion thresholds. The y-axis is the relative abundance (%) of the individual identified as "core" and is color-coded by Phyla/Class (see legend). The x-axis is the proportion of samples in which the core OTU was present at all three sites. [1]Number (and percent of the total) of core OTUs identified.

Table 2.3. Root/rhizosphere soil core bacteriome: OTUs present at all three sites and in 75% of the samples from each site. Average abundance in samples sequenced of each OTU is listed per site.

					Average Abundance		
OTU	Class	Order	Family	Genus	CA	CO	TX
Otu002	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	2.43	14.66	4.70
Otu005	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	4.89	0.91	1.97
Otu007	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	1.84	2.03	0.30
Otu010	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingobium*	0.76	1.73	2.15
Otu015	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	1.09	0.42	1.33
Otu024	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	0.46	0.60	0.24
Otu039	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	0.28	0.26	0.12
Otu067	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Rhizobium*	0.10	0.26	0.28

Table 2.4. Root/rhizosphere soil core bacteriome: OTUs present at all three sites and present in 50% of the samples from each site. Average abundance in samples sequenced of each OTU is listed per site.

					Average Abundance		
OTU	Class	Order	Family	Genus	CA	CO	TX
Otu002	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	2.43	14.66	4.70
Otu005	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	4.89	0.91	1.97
Otu007	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	1.84	2.03	0.30
Otu010	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingobium*	0.76	1.73	2.15
Otu015	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	1.09	0.42	1.33
Otu024	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	0.46	0.60	0.24
Otu039	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	0.28	0.26	0.12
Otu067	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Rhizobium*	0.10	0.26	0.28
Otu029	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Acidovorax*	0.06	0.32	1.59
Otu037	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Agrobacterium*	0.24	0.45	0.18
Otu050	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Brevundimonas*	0.18	0.37	0.04
Otu052	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	0.14	0.35	0.22
Otu056	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingopyxis*	0.12	0.36	0.05
Otu080	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	0.12	0.11	0.09
Otu106	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bosea*	0.11	0.07	0.15
Otu164	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Agrobacterium*	0.04	0.08	0.13
Otu022	Actinobacteria	Actinomycetales	Microbacteriaceae	*Microbacterium*	0.77	0.08	0.05
Otu035	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	0.44	0.18	0.07
Otu058	Betaproteobacteria	Burkholderiales	Alcaligenaceae	*Achromobacter*	0.11	0.18	0.56
Otu102	Alphaproteobacteria	Rhizobiales	unclassified	*unclassified*	0.08	0.15	0.09
Otu207	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Rhizobium*	0.03	0.03	0.14
Otu085	Actinobacteria	Actinomycetales	Micrococcaceae	*Arthrobacter*	0.11	0.15	0.03
Otu231	Actinobacteria	Actinomycetales	Nocardioidaceae	*Aeromicrobium*	0.03	0.05	0.07
Otu170	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Aminobacter*	0.04	0.10	0.05

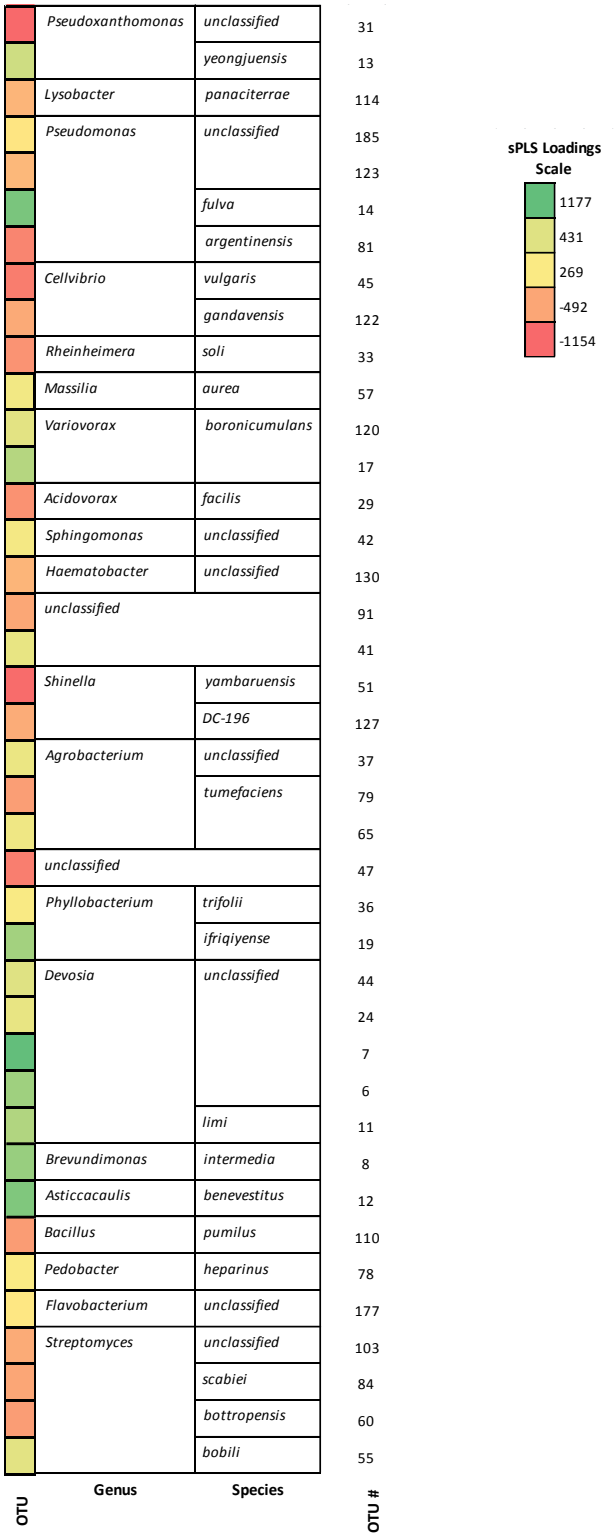

Figure 2.6. Sparse Partial Least Squares Analysis (SPLS) loadings relating root-associated bacterial OTU abundance and tuber yield. SPLS loading scale indicates colors associated with calculated OTU loading numbers. All loadings listed have shown to be significant according to SPLS package in R (Chun and Keles, 2010).

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CHAPTER 3

POTATO TUBER NUTRIENT CONTENT LEVEL IS CLONE-SPECIFIC AND CORRELATES WITH SOIL MICROBIAL DIVERSITY

Chapter Summary

The variability of minerals in potato tubers makes the potato a good candidate for biofortification. Potatoes are a major staple crop for large regions of the world including South America, parts of which have been described as good candidates for the use of biofortified foods. Improving the nutritional value of potatoes can help overcome nutrient deficiencies in developing nations. Soil and tuber samples were collected for 18 potato clones at each of three sites (CA, CO, TX) that are part of the 2011 Western Regional Potato Trials. This study assessed the impact that soil nutrient level, soil bacterial diversity and clone have tuber nutrient content. A predictive model was created for the potato tuber nutrients N, P, K, Zn, Fe, Mn and Cu. All three independent factors (soil nutrients, soil bacterial diversity and clone) were found to have a positive coefficient when employed as predictors of tuber nutrient content. Modifying farm management practices may improve crop nutritional quality for human consumption.

Introduction

Potato is a significant contributor to the human diet worldwide. In parts of South America, potato consumption can be as high as 800 g per day (Burgos et al., 2007) providing a major source of daily recommended mineral intake and protein, with a protein quality similar to that of a whole egg (Desborough and Lauer, 1977; True et al., 1978). Potato produces more dry matter and protein per unit growing area than cereal crops (Bamberg and Del Rio, 2005). Studies have found large cultivar differences for iron in potato, suggesting that biofortification through breeding is possible (Brown et al., 2010; Haynes et al., 2012). While potatoes are not a significant source of calcium, magnesium, or zinc to the human diet, all three minerals have

clonal variability and plants may be bred for higher contents to improve the quality of plant health (Brown et al., 2011, 2012). Improving nutritional quality is desirable as potato consumption in developing countries is steadily increasing. Simultaneously, the world population continues to rise, and by 2050, 2 billion more mouths must be fed as agricultural lands continue to diminish (US Census Bureau, 2012).

Nutrients are transported to tubers by potato plants through either phloem transport, or by direct uptake from the soil across the periderm (Subramanian et al., 2011). Various bacteria provide plants with the nutrients necessary to survive and may be critical in aiding the direct uptake of nutrients from soil. For example, plants can evade manganese deficiency in high oxygen calcareous or manganese toxicity in waterlogged soils because of manganese oxidizing bacteria in the rhizosphere (Babalola, 2010). Legume crop nitrogen needs are met in large part due to members of the bacterial order Rhizobiales (Pagan et al., 1975; Kaneko et al., 2002; Rivas et al., 2002). Plant-bacterial relationships could be exploited to increase desirable crop traits such as higher mineral levels. Although a number of plant growth promoting bacteria have been identified in potato, little is known about their contribution to potato nutrient uptake and tuber quality.

Examining the impact of independent factors (e.g., site, clone) on tuber mineral content can give insight into how to increase the nutritional value of potatoes. Fertilization is an already employed method of increasing plant nutrient contents. For example, adding magnesium to the soil over three years consistently increases potato tuber magnesium content (Klein et al., 1982). However, plants of the same species do not always partition nutrients in similar ways. Two potato cultivars allocate cadmium very differently, Kennebec putting 75% uptake into the tuber, while in Wilwash only 43% is directed to the tuber (Dunbar et al., 2003). Microbes support crop

traits such as yield and disease resistance (Kloepper et al., 1980; Manter et al., 2010), they may also biofortify plants with essential minerals for improved nutritional quality. The objective of this study was to examine site and clone effects on tuber nutrient content in potato tubers using 18 potato clones grown at three sites of the Western Regional Trials (Novy and Stark, 2012). A predictive model for tuber nutrient level was developed to explain the independent factors contributing to potato tuber nutrients.

Materials and Methods

Plant root and rhizosphere soil samples were collected at harvest from the Western Regional Potato Trials in 2011 and stored at -20 °C until processed. Three plants per clone were collected from each of three sites (Tulelake, California; San Luis Valley, Colorado; and Springlake, Texas), shipped fresh, and moved to cold storage (-20 °C). Two to three potato tubers were taken from each plant. At each site, 15 bulk soil samples (5-10 g) were randomly taken from the top three to five inches of soil throughout the potato field, at the same time as plant collection. Random bulk soil samples were pooled, homogenized in a 2 mm sieve, and stored at -20 °C. Bulk soil was sent from each site in triplicate to the Colorado State University Soil, Water, and Plant Testing Laboratory for routine soil analysis. Bacterial DNA was extracted from bulk soil samples (0.5 g) using MoBio PowerSoil-htp kit (catalog # 12955-4, Carlsbad, California) and stored at -20 °C until further processing for pyrosequencing using Roche 454 Junior. Pyrosequence processing and analysis is explained in Chapter 1.

Potato tubers from each plant were washed with deionized water, combined into one sample, frozen in liquid nitrogen and broken into small pieces using a hammer. Samples were then bagged and freeze-dried. The freeze-dried tuber pieces were further ground using an IKA A11 basic grinder (Wilmington, North Carolina). Two grams of ground tuber tissue were

weighed and sent to Colorado State University Soil, Water and Plant Testing Laboratory for mineral testing by wet chemistry. A 2 mL tube, with two chrome steel beads added (BioSpec, catalog #11079123c), was filled approximately halfway with the ground, freeze-dried tuber to be further pulverized for 30 sec in a Mini-Beadbeater-8 (BioSpec, Bartlesville, Oklahoma) and oven dried overnight at 55 ºC. Samples were weighed (3.5-4.5 mg) in duplicate and analyzed for total N and C using a Carlo Erba C/N analyzer (Haake Buschler Instruments, Saddle Brook, New Jersey).

Statistical Analysis

Site differences in soil nutrient contents were analyzed using a one-way ANOVA with Sidak correction (proc GLM, SAS 9.3; SAS Institure, Cary, NC). Site and clone differences in tuber mineral contents were analyzed using a two-way ANOVA with Sidak correction (proc GLM, SAS 9.3). For both tests, dependent variables were tested for equal variance and normality and log-transformed as necessary. A principle component analysis (PCA) is used to simplify data and estimate the correlation of variables, and is a pragmatic first step for large datasets (Wold et al., 1987). The PCA based on tuber nutrient contents was performed with the aid of PC-ORD Version 6 (McCune and Grace, 2002). Associations between the principal components and soil characteristics (i.e., edaphic and microbial community) were visualized by biplot, and only those characteristics with a correlation coefficient greater than 0.35 with either axis are presented. Predictive models of tuber nutrient contents were developed using proc MIXED in SAS 9.3. For each tuber nutrient, three different models were tested, the independent variables were as follows: (model 1) soil nutrient content; (model 2) soil nutrient content and soil microbial diversity; and (model 3) soil nutrient content, soil microbial diversity, and a clone coefficient. The soil microbial diversity parameter was the "true" diversity index (Jost, 2006) of

the bulk soil from each site (see Chapter 1). "True" diversity was used because the formula for Shannon's diversity index (*H*) contains a natural logarithm, this means that the scale on which diversity indices are reported is not linear (Hill, 1973). To directly compare diversity measurements, Jost recommends converting *H* by raising base *e* to the power of *H*, or e^H. A unique, dimensionless, site-independent clone coefficient was calculated for each nutrient by dividing the overall clone average tuber nutrient content ($n = 9$) by the overall study average tuber nutrient content ($n = 162$).

Results

Bulk soil samples differed significantly from one another ($p < 0.001$) in all measurements except electrical conductivity, percent organic matter, zinc and copper (Table 3.1). Soil pH was significantly different among all sites, ranging from strongly acidic (5.29 in CA) to neutral (7.10 in TX) and slightly alkaline (7.55 in CO) (Table 3.1) (Soil Survey Division, 1993). Potato tuber nutrients differed significantly at each site ($p < 0.001$) with the exception of iron and boron. Tuber phosphorus and potassium were different among sites, CA and TX in the same statistical group but higher than CO for phosphorus, and CA and CO grouping together but higher than TX for potassium (Table 3.2). Clonal differences ($p < 0.05$) were seen in all measurements except carbon, manganese and copper (Table 3.3). Nutrient-site averages for soil and tuber nutrients exhibited different patterns; for example, tuber zinc was highest in TX followed by CA then CO; however, soil zinc was highest in CA, followed by CO and TX (Figure 3.1). No one site was consistently highest or lowest for all of the nutrient measurements in either soil or tubers.

Principal component analysis (PCA) seeks to combine independent variables (in this case tuber minerals) into linear combinations (i.e. axis) in an effort to explain as much variation in the data as possible. Edaphic and microbial features were correlated with the data and visually

depicted as a biplot (red vectors in Figure 3.2). The first PCA axis explained 44.0% and the second PCA axis explained 16.5% of the variation in the tuber nutrient data (Figure 3.2). Interestingly, only two soil factors, Cu and the bulk soil "true" bacterial diversity were correlated ($r > 0.35$) with either of the first two PCA axes. Because of this visual correlation, bulk soil diversity was considered as a potential predictor of tuber nutrient contents, as described below.

Mixed model analysis in SAS 9.3 uses independent predictors, which may be nested (e.g., clones within a site), to express a dependent variable (e.g., tuber nutrient) as a function of the predictors (Suzuki and Sheu, 1999). The Akaike Information Criterion (AIC) was used to confirm relative quality of model fit as each independent variable was added to the model. A more negative AIC denotes better model fit (Akaike, 1974). The first model solely used soil nutrient to explain tuber nutrient, AIC values for nitrogen, phosphorus and potassium were -25.1, -14.1, -109.4, respectively (Figure 3.3). Observed average tuber nutrient versus predicted tuber nutrient was a poor fit, particularly for TX nitrogen and CO and TX phosphorus. Model 2 adds bulk soil "true" diversity as an independent variable to soil nutrient. The AIC is more negative than Model 1 for tuber N, P, and K. The increased fit of Model 2 was largely associated with a better fit for all nutrients for the TX samples, a better fit for phosphorous for the CO sample. To account for clone variation, Model 3 incorporated the clone coefficient previously calculated (Table 3.5). This final model (Model 3) was a better fit than Model 2; the AIC was further improved from -69.6, -99.6, -151.5 to -105.1, -135.0, -171.8 for N, P, and K (Figure 3.3). All independent predictors were significant ($p < 0.01$) for each tuber mineral measured, except for tuber Fe where neither soil Fe nor "true" diversity were significant (Table 3.4). In addition to the *p*-value the partial correlation for each independent variable was reported in Table 3.4, this value describes the relative effect each independent variable has on the predicted tuber nutrient. Partial

correlation coefficients for each independent predictor help explain the relative influence each factor has on tuber nutrient level. Soil nutrient and soil bacterial diversity (exp(H)) have similar correlation coefficients for each nutrient, soil bacterial diversity correlation coefficient is higher than soil nutrient for potassium, manganese and copper. Clone correlation coefficient is lower than either two of the other predictors, in the case of phosphorus, the clone correlation coefficient is 0.330 while soil P and soil bacterial diversity are 0.830 and 0.806, respectively.

Discussion

Staple foods such as potato feed many populations of the world. Mineral biofortification of crops seeks to decrease human disease and mortality through the provision of foods with high quality nutrients (Bouis and Welch, 2010). The potato is a staple food for much of South America, where some locations have been identified as good candidates for implementation of staple crop biofortification (Burgos et al., 2007; Zapata-Caldas et al., 2009). Potato has been recommended as a worthy candidate for biofortification; providing a food of higher nutritional value to areas that already consume potatoes can increase the likelihood of lowering disease due to malnutrition (Haynes et al., 2012).

To understand the variability in site and clones, potato and soil samples were collected from three sites (CA, CO and TX) and nutrient levels in tubers along with soil features were quantified. Soils across all three sites vary in nutrient levels; in addition, nutrient levels in different potato clones vary. Consistently, sites with the highest soil nutrients did not necessarily produce tubers with the highest nutrient levels. A principle component analysis (PCA) helped visualize the relationship of measured edaphic and microbial components to potato tuber nutrients. Interestingly, soil bulk diversity was correlated with tuber nutrients. Better plant health is seen in soils with higher bacterial diversity, and nutrient use efficiency by plants is

driven by nutrient cycles which are driven by soil microorganisms (Clarholm, 1985; Brussaard et al., 2007). High bacterial diversity may provide the agro-ecosystem with a sufficient community to provide services for plant nutrient uptake.

Plants of the same species can partition elements differently; of two potato cultivars, Kennebec stores 75% of cadmium intake in the tuber while in Wilwash, 43% is allocated to the tuber (Dunbar et al., 2003). Variation for other nutrients is seen in sweet potato cultivars where amino acid contents differ (Purcell et al., 1972). Thus a clone coefficient was calculated to account for the variation in tuber nutrients. This coefficient was used as a predictor for the final model and is calculated by averaging clone level of each nutrient over all three sites and dividing by the average content of the same nutrient of all clones at all three sites. The clone coefficient value cannot explain how the disparity in plant cultivar nutrient uptake occurs, for example if plant genetics is the driving force; however it is possible the reason for differential uptake and partitioning is due to biological processes, such as cultivar-specific microbial communities. Manter et al. (2010) examined 20 clones where microbial community differences were associated with plant biomass. Whether nutrient acquisition is more highly dependent on a bacterial or plant process is not as important as understanding that the two communities are intertwined to the point where a change in one community, either plant or bacterial, will affect the other community. By including soil nutrient level, soil microbial diversity and potato clone variations, the results presented here acknowledge the impact of three major contributors to crop success.

The influence of soil nutrient, soil bacterial diversity and cultivar effect on tuber nutrient contents are presented in a statistical model, which shows any of the three independent predictors (soil nutrient level, bacterial diversity, and clone) can support an increase in tuber nutrients. The

first two models investigated had fewer predictors (model 1: soil nutrient levels, model 2: soil nutrient and bulk soil bacterial diversity) and fit the data poorly, the final model fits the data well and has the lowest AIC (best fit) of the three models tested. Partial correlation coefficients explain the normalized effect of each independent variable on tuber nutrient contents. Partial correlation coefficients for bulk soil "true" diversity and soil nutrient levels were often similar; however, some nutrients have a higher partial correlation coefficient for soil bacterial diversity than soil nutrient level: K, Mn and Cu. That these coefficients are similar for soil nutrient level and soil bacterial diversity indicates that the two predictors are of near equal importance to the contribution of nutrient levels in potato tubers.

Fertilizers, lime and soil conditioners together comprise one of the top three average expenses a farm incurs, and the cost for these inputs rose 20.6% from 2010 to 2011 (National Agricultural Statistic Service, 2012). Increasing soil bacterial diversity, just one independent factor, may benefit agriculture by supporting acquisition of multiple nutrients in food while lowering multiple fertilizer inputs. In addition to expenses saved, increasing bacterial diversity to replace a portion of fertilizer will lead to less contamination of ecosystem services that are provided by soil, such as water filtration (Tilman et al., 2002). This system could be a cost effective, sustainable way to support plant and human health. Bacterial diversity can support plant nutrient levels and be considered as an ecosystem service that helps to feed crops which feed the world.

Figures and Tables

Table 3.1. Average soil nutrients at each site. Each measurement is an average of three replicates taken from each site (state). For each measurement, values with different letters are significantly different. *EC*: Electrical Conductivity, *OM*: Organic Matter, *NO_3-N*: Soil Nitrate. [a]mmhos/cm, [b]%, [c]ppm.

State	pH	EC[a]	OM[b]	NO_3-N[c]	P[c]	K[c]	Zn[c]	Fe[c]	Mn[c]	Cu[c]
CA	5.29 c	0.5 a	2.93 a	41.9 a	23.7 a	367.2 a	5.13 a	38.1 a	5.3 a	5.2 a
CO	7.55 a	0.5 a	2.9 a	13.5 b	6.8 b	197.4 b	3.4 a	3.2 b	1.8 b	9.4 a
TX	7.1 b	0.433 a	0.9 a	13.9 b	4.2 c	127.6 c	1.5 a	3.0 b	1.5 b	2.8
p-value	<.0001	0.8382	0.2885	<.0001	<.0001	0.0002	0.0989	<.0001	0.0005	0.5471

Table 3.2. Average potato tuber nutrient contents at three different sites. Each measurement is the average of 18 potato clones, 3 replicates per clone. Values are back-transformed LSMean. For each measurement, values with different letters are significantly different ($\alpha = 0.05$, Sidak corrected). [a]%, [b]ppm.

State	C[a]	N[a]	P[a]	K[a]	Ca[a]	Mg[a]	Na[a]	Fe[b]	Mn[b]	Zn[b]	Cu[b]	B[b]
CA	44.3 a	2.11 a	0.392 a	1.29 b	0.039 b	0.123 a	0.019 b	74.5 a	8.40 b	17.1 b	7.81 a	4.51 a
CO	44.2 a	1.38 b	0.201 b	1.26 b	0.047 a	0.106 b	0.010 c	76.9 a	7.80 b	12.0 c	5.31 b	4.20 a
TX	43.5 b	2.25 a	0.406 a	1.92 a	0.055 a	0.125 a	0.116 a	84.5 a	10.9 a	27.9 a	6.10 b	4.33 a
p-values												
State	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.355	<0.001	<0.001	<0.007	0.622

Table 3.3. Average tuber nutrient content for 18 cultivars averaged across all three sites. Three replicates per clone at each of the three sites, *n*=9 per clones. Values are back-transformed LSMeans. For each nutrient, values with different letters are significantly different ($\alpha = 0.05$, Sidak corrected). [a]%, [b]ppm.

Cultivar	C[a]	N[a]	P[a]	K[a]	Ca[a]	Mg[a]	Na[a]	Fe[b]	Mn[b]	Zn[b]	Cu[b]	B[b]
A01010-1	44.4 a	1.82 abcd	0.326 ab	1.37 a	0.065 ab	0.132 ab	0.043 a	128.6 ab	9.78 a	18.7 abcde	6.23 a	5.09 a
A01025-4	43.8 a	2.00 abc	0.338 ab	1.46 a	0.072 a	0.138 a	0.040 ab	137.0 a	9.53 a	19.2 abc	5.76 a	4.88 a
A99331-2R/Y	44.4 a	1.97 abcd	0.326 ab	1.30 a	0.034 bcd	0.117 ab	0.018 c	65.7 abc	7.67 a	22.7 ab	6.51 a	4.87 a
A99433-5Y	43.6 a	1.75 abcd	0.366 ab	1.47 a	0.046 abc	0.122 ab	0.029 abc	81.6 abc	9.36 a	18.6 abcde	6.73 a	5.63 a
AC99375-1RU	44.0 a	1.37 d	0.276 b	1.41 a	0.059 ab	0.113 ab	0.040 ab	98.7 abc	9.70 a	13.6 de	6.61 a	4.55 a
ATTX98453-6R	43.8 a	2.31 a	0.333 ab	1.41 a	0.038 bcd	0.115 ab	0.025 abc	53.0 c	9.12 a	22.1 ab	5.87 a	4.78 a
ATTX98510-1RY	44.3 a	1.69 bcd	0.287 ab	1.40 a	0.026 d	0.113 ab	0.020 abc	70.1 abc	7.39 a	13.2 e	6.33 a	3.88 a
CO00291-5R	43.8 a	1.89 abcd	0.329 ab	1.71 a	0.044 abcd	0.122 ab	0.027 abc	60.7 bc	8.03 a	21.1 ab	6.83 a	4.52 a
CO01399-10/PY	43.6 a	1.88 abcd	0.329 ab	1.63 a	0.038 bcd	0.116 ab	0.024 abc	74.4 abc	8.86 a	20.1 abc	6.09 a	5.63 a
CO99053-3RU	44.3 a	1.70 bcd	0.278 b	1.43 a	0.064 ab	0.107 b	0.037 abc	93.7 abc	10.5 a	14.5 cde	5.54 a	3.54 a
CO99100-1RU	44.3 a	2.09 abc	0.305 ab	1.34 a	0.059 ab	0.119 ab	0.037 abc	99.5 abc	9.94 a	19.1 abcd	5.81 a	4.19 a
CO99256-2R	43.8 a	2.03 abc	0.385 a	1.62 a	0.032 cd	0.122 ab	0.021 bc	68.0 abc	7.92 a	20.9 ab	7.89 a	3.83 a
COTX01403-4R/Y	43.9 a	1.98 abc	0.335 ab	1.45 a	0.032 cd	0.120 ab	0.019 c	63.0 abc	8.08 a	19.1 abcd	5.97 a	4.23 a
Dark Red Norland	44.1 a	2.18 ab	0.370 ab	1.56 a	0.044 abcd	0.120 ab	0.021 bc	58.9 bc	10.6 a	23.1 a	7.09 a	4.06 a
Ranger Russet	44.4 a	1.57 cd	0.299 ab	1.38 a	0.064 ab	0.107 b	0.033 abc	86.0 abc	8.14 a	14.3 cde	6.79 a	4.08 a
Red LaSoda	43.7 a	1.92 abcd	0.343 ab	1.57 a	0.037 bcd	0.113 ab	0.018 c	51.9 c	7.78 a	21.6 ab	7.59 a	4.13 a
Russet Norkotah	44.4 a	1.83 abcd	0.271 b	1.32 a	0.071 a	0.103 b	0.031 abc	107 abc	10.2 a	14.9 bcde	5.08 a	3.63 a
Yukon Gold	43.9 a	1.9 abcd	0.277 b	1.50 a	0.036 bcd	0.116 ab	0.020 c	64.0 abc	8.69 a	14.8 bcde	6.22 a	3.78 a
p-value	0.140	<0.001	<0.001	0.009	<0.001	0.006	<0.001	<0.001	0.486	<0.001	0.989	0.058

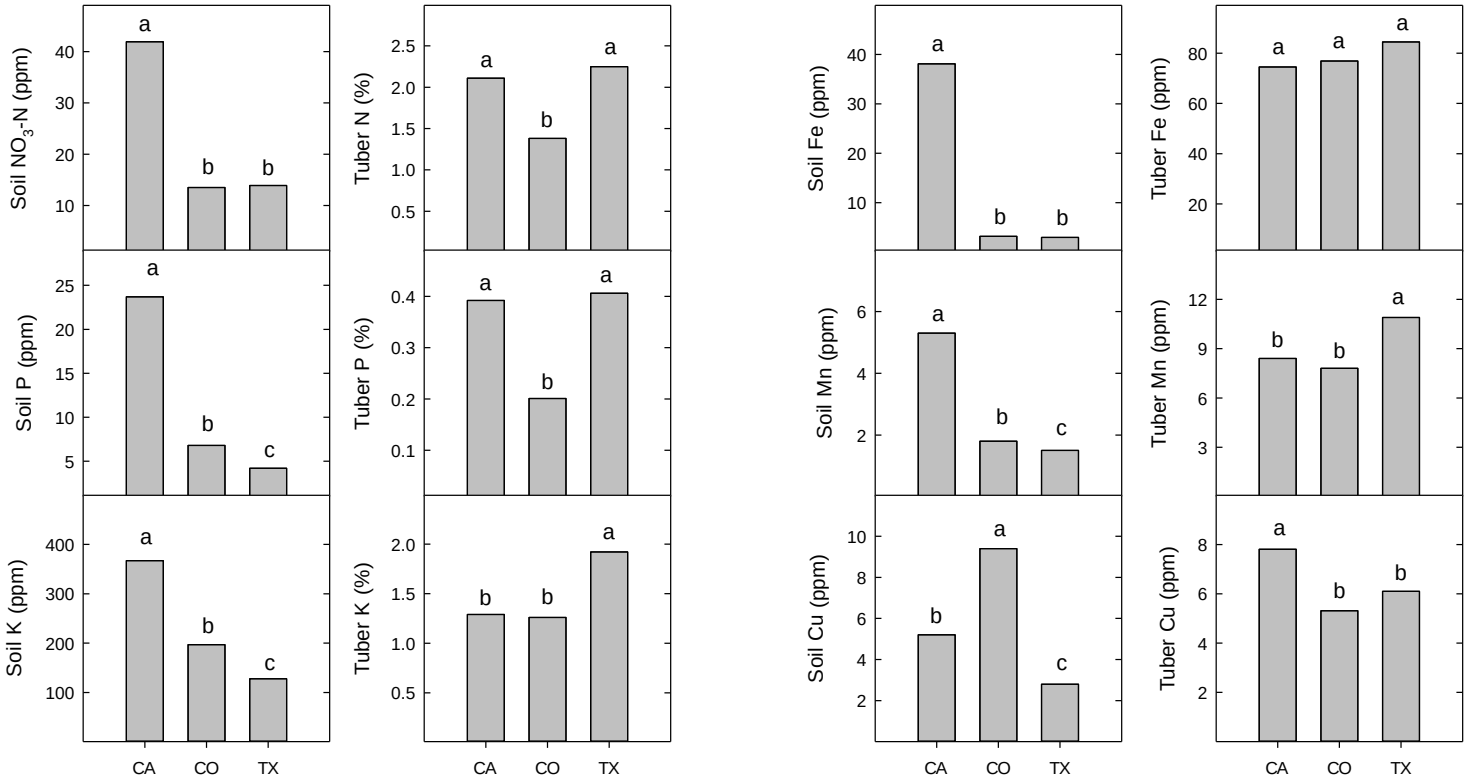

Figure 3.1. Soil and tuber nutrients from potatoes grown at three sites. Soil nutrients are the average of 3 bulk samples per site, tuber nutrients are the average of 18 potato clones at each site site (x-axis).

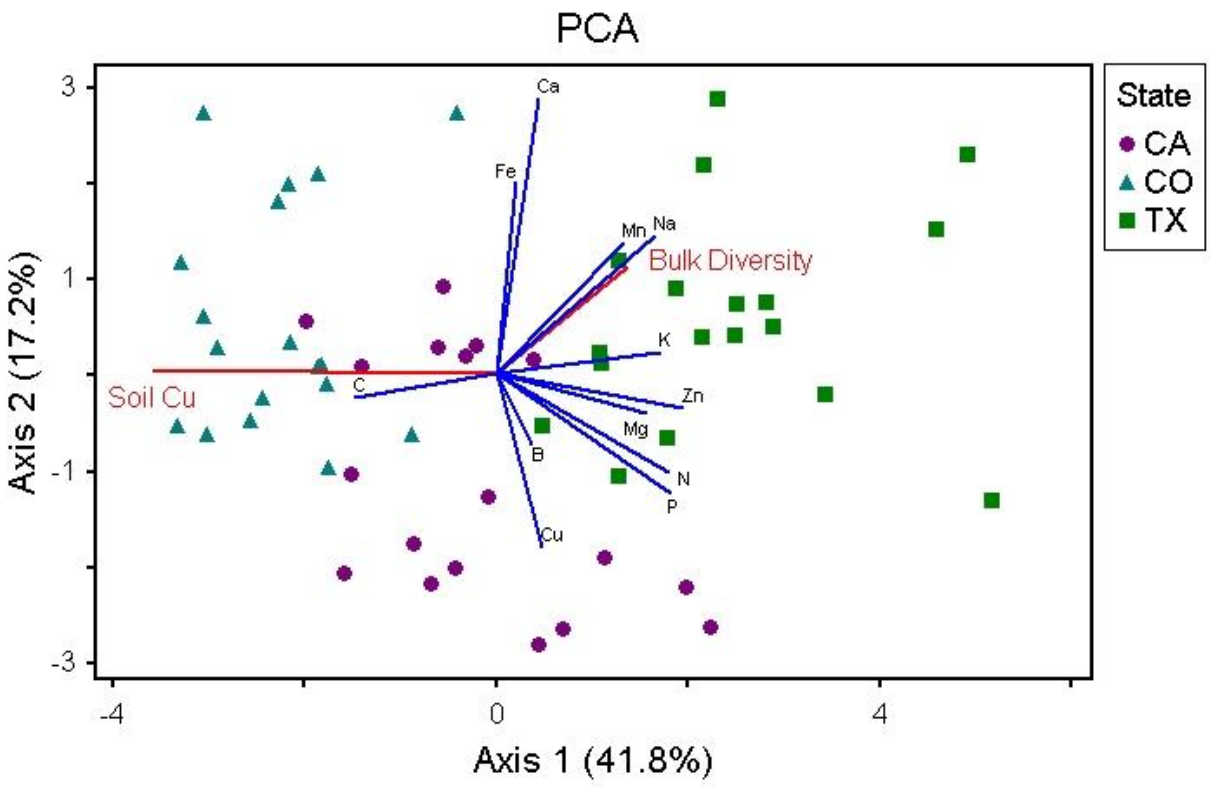

Figure 3.2. Principle Components Analysis using soil and tuber nutrients. Independent factors (red vectors) have at least a 0.35 correlation coefficient to blue tuber nutrients shown, the length of the each vector is relative to the correlation coefficient of that factor. *Bulk Diversity*: Bulk soil "true" bacterial diversity.

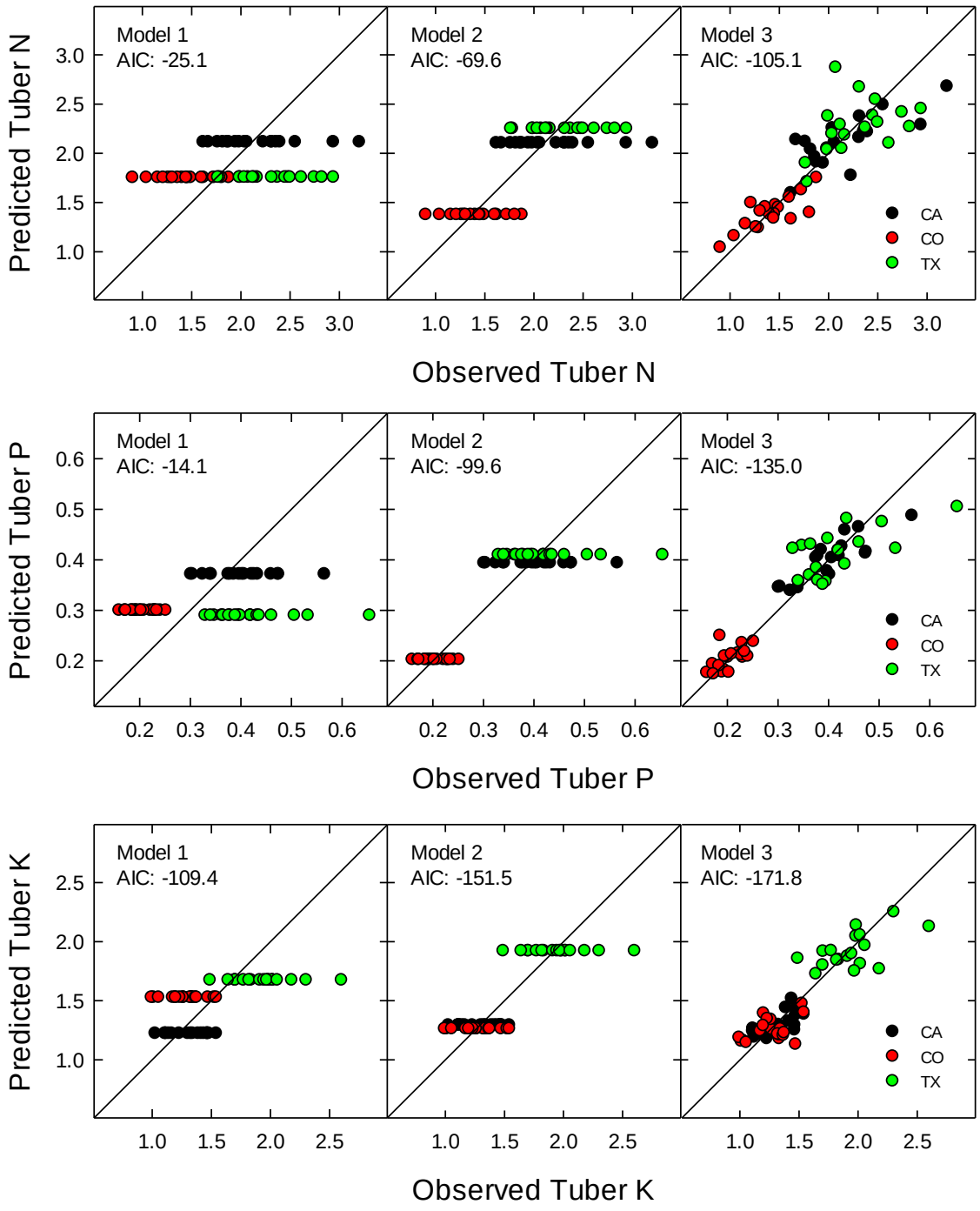

Figure 3.3. Observed tuber nutrients versus predicted tuber nutrients for each model. Model 1 independent variable: soil nutrient, Model 2 independent variables: soil nutrient and bulk soil "true" bacterial diversity, Model 3 independent variables: soil nutrient, bulk soil "true" bacterial diversity, clone coefficient. AIC values top corner of each square measure model fit, more negative is a better fit.

Table 3.4. Final model for predicting tuber nutrient contents for 18 potato cultivars across three sites. [a]Type II partial correlation.

Nutrient	Parameter Estimates					Model Fit		
	Name	Estimate	SE	Partial Corr[a]	Pr > |t|	Adj. R^2	AIC	Pr > F
ln(N)	Intercept	-1.944	0.182	.	<0.001	0.695	-105.1	<0.001
	Soil NO_3	0.0337	0.00250	0.644	<0.001			
	Soil exp(H)	0.00233	0.000192	0.588	<0.001			
	Clone	1.04	0.138	0.365	<0.001			
ln(P)	Intercept	-4.89	0.186	.	<0.001	0.841	-135.0	<0.001
	Soil P	0.104	0.00460	0.830	<0.001			
	Soil exp(H)	0.00474	0.000227	0.806	<0.001			
	Clone	1.02	0.139	0.330	<0.001			
ln(K)	Intercept	-3.058	0.328	.	<0.001	0.691	-171.8	<0.001
	Soil K	0.00533	0.000651	0.426	<0.001			
	Soil exp(H)	0.00384	0.000371	0.538	<0.001			
	Clone	0.964	0.183	0.230	<0.001			
ln(Zn)	Intercept	22.9	1.22	.	<0.001	0.823	-78.1	<0.001
	Soil Zn	-3.55	0.201	0.729	<0.001			
	Soil exp(H)	-0.0285	0.00168	0.714	<0.001			
	Clone	1.06	0.0932	0.523	<0.001			
ln(Fe)	Intercept	3.27	0.244	.	<0.001	0.310	163.6	<0.001
	Soil Fe	0.00150	0.00474	0.000765	0.746			
	Soil exp(H)	0.000414	0.000451	0.00645	0.346			
	Clone	0.929	0.123	0.317	<0.001			
ln(Mn)	Intercept	0.224	0.307	.	0.470	0.241	73.7	<0.001
	Soil Mn	0.135	0.0352	0.0935	<0.001			
	Soil exp(H)	0.00177	0.000349	0.153	<0.001			
	Clone	1.00	0.228	0.120	<0.001			
ln(Cu)	Intercept	1.40	0.389	.	<0.001	0.131	194.4	<0.001
	Soil Cu	-0.0473	0.0151	0.0644	0.002			
	Soil exp(H)	-0.000820	0.000229	0.0825	<0.001			
	Clone	0.990	0.367	0.0488	0.008			

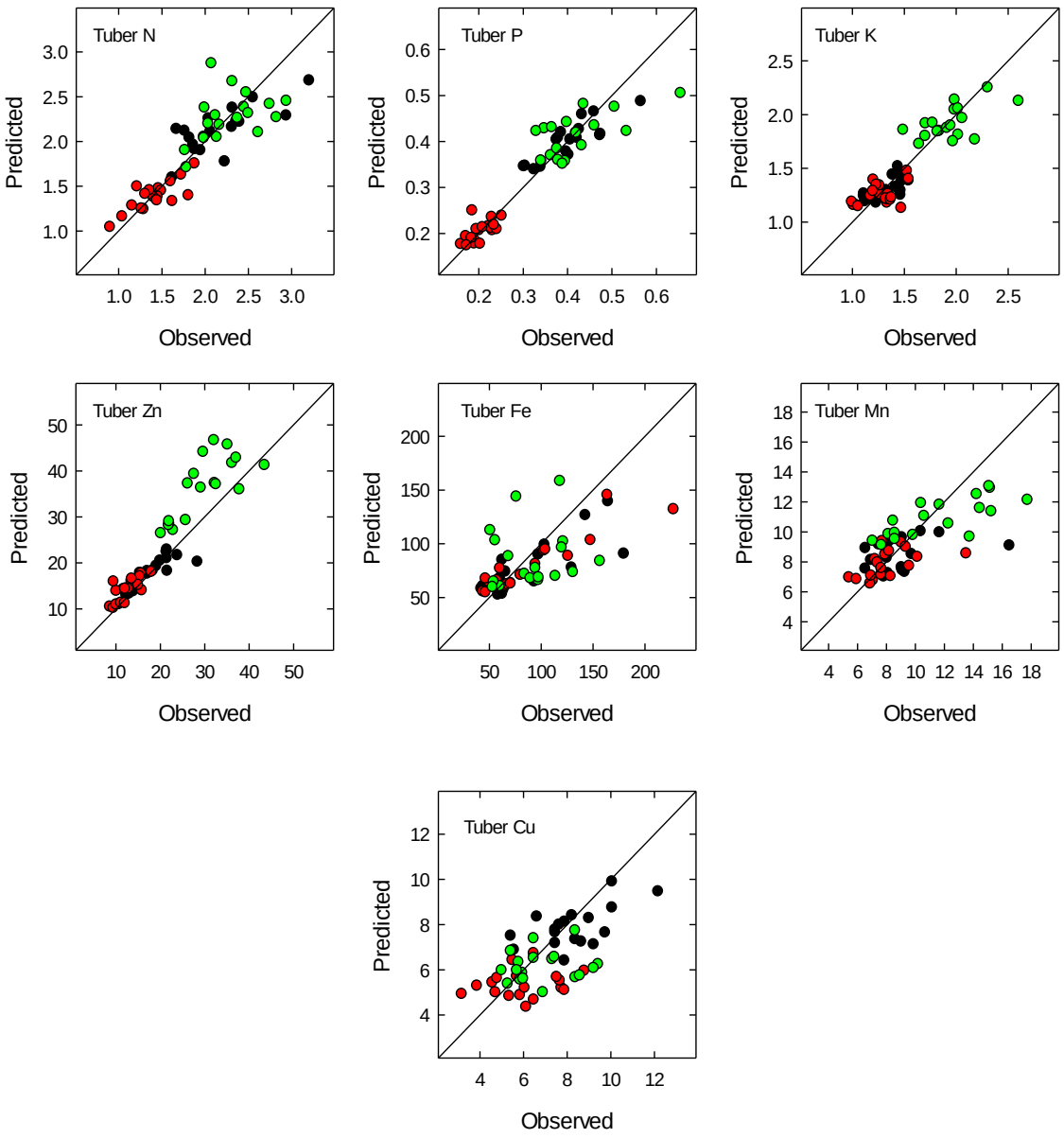

Figure 3.5. Observed tuber mineral versus calculated predicted tuber mineral for Model 3. Independent variables for Model 3: soil nutrient, bulk soil "true" bacterial diversity, clone coefficient. Each color is a different site, *black:* CA, *red:* CO, *green:* TX.

Table 3.5. Clone coefficients. Each coefficient is calculated by dividing the average clone nutrient level over 3 sites (n=9) by the average nutrient level of all clones at 3 sites (n=162). These are the clone coefficients used as an independent variable in Model 3.

Cultivar	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	B	Mo
A01010-1	0.966	1.016	0.935	1.359	1.123	1.539	1.583	1.091	1.016	0.975	1.154	0.270
A01025-4	1.063	1.054	1.000	1.505	1.174	1.431	1.687	1.063	1.042	0.902	1.108	0.478
A99331-2R/Y	1.047	1.016	0.891	0.711	0.996	0.644	0.808	0.856	1.233	1.019	1.106	3.134
A99433-5Y	0.929	1.141	1.003	0.962	1.038	1.038	1.005	1.045	1.008	1.055	1.279	1.910
AC99375-1RU	0.730	0.861	0.961	1.233	0.962	1.431	1.215	1.082	0.739	1.035	1.032	2.334
ATTX98453-6R	1.229	1.038	0.967	0.794	0.979	0.895	0.652	1.017	1.200	0.920	1.085	0.519
ATTX98510-1RY	0.898	0.895	0.959	0.544	0.962	0.716	0.863	0.824	0.714	0.991	0.880	0.404
CO00291-5R	1.002	1.026	1.167	0.920	1.038	0.966	0.747	0.896	1.147	1.070	1.026	1.953
CO01399-10/PY	0.999	1.026	1.113	0.794	0.987	0.859	0.916	0.988	1.091	0.954	1.277	1.872
CO99053-3RU	0.904	0.867	0.977	1.338	0.911	1.324	1.154	1.174	0.784	0.868	0.803	0.858
CO99100-1RU	1.113	0.951	0.916	1.233	1.013	1.324	1.225	1.109	1.036	0.910	0.951	0.276
CO99256-2R	1.077	1.200	1.108	0.669	1.038	0.751	0.837	0.884	1.136	1.235	0.869	1.225
COTX01403-4R/Y	1.050	1.045	0.990	0.669	1.021	0.680	0.775	0.901	1.039	0.934	0.961	0.654
Dark Red Norland	1.159	1.154	1.067	0.920	1.021	0.751	0.725	1.182	1.253	1.110	0.923	0.804
RangerRusset	0.832	0.932	0.942	1.338	0.911	1.181	1.059	0.909	0.775	1.063	0.926	0.315
Red LaSoda	1.021	1.069	1.072	0.774	0.962	0.644	0.639	0.868	1.172	1.190	0.938	0.402
Russet Norkotah	0.971	0.845	0.905	1.484	0.877	1.109	1.320	1.140	0.811	0.795	0.824	0.359
Yukon Gold	1.011	0.864	1.026	0.753	0.987	0.716	0.788	0.970	0.803	0.974	0.859	0.234

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CHAPTER 4

DISCUSSION

To better understand the utility of the soil microbiome for crop success, this research examined microbial communities from bulk and root/rhizosphere soil of 18 potato clones at three sites. Identifying microbes or microbial characteristics associated with crop improvement gives growers and researchers another option besides chemical inputs to improve crop characteristics. This research examined the relationship soil bacteria have with crop yield and nutrient acquisition in potato tubers.

Chapter 2 defined a "core bacteriome" of root/rhizosphere soil for multiple potato clones across three sites. The term "core bacteriome" does not have a confirmed definition, so multiple parameters were placed on the data to create a core set of bacteria using presence/absence in samples. Multiple levels of a core were defined, thus placing different parameters on a dataset to describe a core yielded very different "core bacteriomes", even within the same dataset. A different manner of examining the core bacteria to potato roots/rhizosphere soil was then employed. By using the SPLS statistical program in R, bacteria that were commonly associated with yield among all sites were extracted by their loadings (i.e., impact). Only samples with significant loadings were reported, thus a list of bacteria was generated that explained either a positive or negative relationship with yield. Members of the bacterial order Rhizobiales are well represented on the list, and most have a positive correlation to potato yield. While these bacteria have not been commonly associated with potato yield, they may have an important relationship not previously studied with potato plants.

Chapter 3 examined three predictors (soil nutrient, bulk soil bacterial diversity, and clone effect) for bacterial nutrient content of each N, P, K, Zn, Fe, Mn and Cu. Each predictor was

found to be a significant contributor to potato nutrient content. Interestingly the highest contributors, bacterial diversity and soil nutrient level, have similar partial correlation coefficients, meaning that they have equal capacity to increase soil nutrient levels in potato tubers in the model presented. Perhaps soil bacterial communities can support biofortification of crops.

This research highlights the potential of the soil bacterial community to increase crop yield and potato nutrient levels. Several individual bacteria were correlated to potato yield and bulk soil bacterial diversity was correlated to increased tuber nutrients. This gives farmers two options to support growth and nutrients, depending on goals and management style. The use of chemical inputs may be decreased if soil bacteria are exploited to their full potential.

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APPENDIX

Table A.2.1 Potato clone root/rhizosphere samples sequenced and the total reads observed from Roche GS Junior Pyrosequencer. One replicate was sequenced per clone/site. *Dashes*: no sample sequenced from that clone/site.

Clone/Cultivar	Total Reads		
	CA	CO	TX
A01010-1	3237	1984	---
A01025-4	1814	2206	---
A02060-3TE	3368	4774	---
A98345-1	3107	2880	---
A99331-2R/Y	3679	2085	---
A99433-5Y	3124	5714	---
AC99375-1RU	2088	3899	---
AOTX96265-2RU	6763	3311	---
ATCO0293-1W/Y	5465	1485	2663
ATTX01178-1R	3206	2992	2542
ATTX098453-6R	10700	2427	2199
ATTX98510-1R/Y	8313	1483	1860
CO00291-5R	1832	2540	---
CO01399-10P/Y	2846	2150	---
CO99053-3RU	3080	2409	---
CO99053-4RU	3359	---	3128
CO99076-6R	3857	1433	2158
CO99100-1RU	5956	---	2278
CO99256-2R	4147	3790	1984
COTX01403-4R/Y	2480	3066	2375
Norland	12467	3949	---
RangerRusset	1835	---	2189
RedLaSoda	2733	1073	---
RussetBurbank	1755	4372	2155
RussetNorkotah	3924	4626	---
YukonGold	1998	3990	---
Bulk Soil.1	4534	1940	2910
Bulk Soil.2	2905	2133	2467