THESIS

EFFECT OF PHOSPHORUS FERTILIZATION ON RHIZOSPHERE MICROBIOME OF CROPS

Submitted by

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ABSTRACT

EFFECT OF PHOSPHORUS FERTILIZATION ON RHIZOSPHERE MICROBIOME OF CROPS

Recent studies in plant-microbe interactions have revealed the importance of the rhizosphere microbiome in agriculture. However, little is known about the impact of fertilization on the rhizosphere and its associated microbial communities. This thesis investigates whether phosphorus (P) fertilizer has led to a shift in bacterial community composition and functions in both cultivated and non-cultivated plants. Two independent greenhouse experiments were conducted to evaluate P impacts. The first study explored the effects of low (0 and 50 kg ha^{-1}) and higher P levels (101 and 192 kg ha^{-1}) of triple super phosphate (0-45-0) amendments on soil microbial community composition associated with the rhizosphere of blueberry plants. The abundance of soil bacteria with phosphatase genes was also tested. The second experiment used a gradient of domesticated potato plants (modern cultivars, landrace and wild) to evaluate the effect of P addition on plant biomass and bacterial communities associated with the potato rhizosphere. Further, the study aimed to detect the most abundant microbial taxa, shared and unique, across six genotypes of *Solanum* genera. Four tuber-bearing and two non-tuber bearing potatoes were used in this study. Tuber-bearing included *Solanum tuberosum* subsp. *tuberosum* (a direct progenitor of modern potatoes) and the potato cultivars 'Red Norland', 'Yukon Gold' and 'Russet Burbank'. The non-tuber bearing potatoes included *Solanum bulbocastanum* and *Solanum tuberosum* subsp. *tuberosum.* Plants were grown in soils collected from an agricultural field where cover crops were previously cultivated. Three levels of phosphorus were applied (0,

67, 133 kg ha^{-1}) during the experiment. Rhizosphere soil was collected and analyzed by amplicon sequencing targeting 16S rRNA gene. Our results showed that potato genotype is the main driver of microbial community composition, followed by fertilizer level. Non-tuber bearing potatoes were different from tuber-bearing potatoes and showed a higher degree of dissimilarity in microbial taxa compared to others. Additionally, a shift in bacterial abundance within the community was observed in response to high P levels. Xanthomonadacea and Alteromonadacea were the two families consistently increase or decrease (respectively) in response to incremental P levels. Interestingly, the latter was only present in non-cultivated potato plants, this family could be an important microbial member that has been lost with cultivation.

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CHAPTER 1: LITERATURE REVIEW

Introduction

The configuration of the communities living in the rhizosphere environment are affected by several host and external factors such as soil type, plant species and major soil disturbances such as continuous mineral fertilization. The composition of rhizosphere communities will determine their functionality and therefore its effect on plant-host health. Environmental challenges faced by the plant can hamper plant function and productivity. To some extent, these challenges can be controlled by management practices. For this reason, it is crucial to understand the importance of fertilization as a soil disturbance factor and its impact on microbial communities associated with plant root. In this first chapter the dynamics of rhizosphere microorganisms in the soil are presented, the importance of phosphorus in the soil and plant is highlighted and the most recent reports on the impacts of the domestication process on soil microorganisms are discussed.

The role of the rhizosphere microbiome on plant nutrition and health

The rhizosphere has been defined, more than a century ago, as the zone around the roots where microorganisms that are intimately linked to roots of plants are located (Hinsinger and Marschner, 2006). Elemental plant functioning processes such as plant nutrient uptake and protection against pathogens attack take place in this hotspot. (Berendsen et al., 2012). Thus, the rhizosphere microbiome refers to the ecological community of commensal, symbiotic and pathogenic microorganisms that share this environment (Lederberg and McCray 2001). Among the microbial communities populating the rhizosphere, bacteria constitute 90-95% of the whole

population (Timmusk et al. 2017). This is the reason why this thesis is focused strictly on the study of bacterial communities.

Plant root microbiome assemblage

The soil location where the plant is established determines the indigenous biota to which the plants are exposed and that develops as the rhizosphere microbiome. Further, these soil microbial communities are structured by physico-chemical properties of the soil and biogeochemical processes (Fierer and Jackson, 2006). Lastly, the plant genotype defines which members of this reservoir of microorganisms can grow and thrive in the rhizosphere (Philippot et al., 2013). Plant genotypes influence the composition of the rhizosphere microbiome by secreting rhizo-deposits such as mucilage, sloughed-off root cells and exudates varying in composition and amount (Bais et al., 2006). Root exudation mediates communication between plants and soil (Walker et al., 2003; Rovira 1969) through specific root-secreted metabolites capable of triggering multiple responses in different soil microorganisms. For instance, banana root exudates containing organic acids (e.g. malic acid, fumaric acid) can induce chemotaxis and biofilm formation in the PGPR (Plant Growth Promoting Rhizo-Bacteria) *Bacillus amyloliquefaciens.* (Yuan et al., 2015). Likewise, other metabolites secreted into the soil have been found to solubilize nutrients for the plants. For instance, *Lupin* spp. is capable of utilizing P from a non-labile pool by releasing citric acid or citrate ions in sufficient quantities to lower the rhizosphere pH, thus enhancing the movement of P (Braum and Helmke, 1995).

Soil microbes can significantly influence the nutrient status of plants. They mediate plant growth promotion by nutrient mobilization, mineralization, soil organic matter decomposition, nitrogen fixation, and phosphate and potassium solubilization (Kaul et al., 2018). Some well-known examples are nodule inducing *Rhizobium* sp. and free-living *Azotobacter* sp. both

nitrogen fixing bacteria. Phosphorus solubilizing bacteria such as *Pseudomonas* sp. and *Enterobacter* sp. as well as *Thiomonas* sp., *Geobacter* sp. and *Algaligenes* sp. enhance solubilization of sulfur, manganese and iron; respectively (Suleman et al., 2018; Osorio-Vega, 2007).

The phosphorus legacy in agricultural soils

For more than 50 years, farmers around the globe have been encouraged to apply phosphorus (P) fertilizers to improve soil fertility and meet crop nutrient demand (Withers et al., 2014; 2015). Unfortunately, the high fixing capacity of P in many soils and the low P use efficiency of most crops have contributed to P accumulation in the soil, often in forms unavailable to plants (Withers et al., 2001). This P accumulation in soils is called P legacy, which can be calculated by considering the differences of P inputs (mineral P fertilizer, atmospheric deposition and weathering) and outputs (surface run-off, subsurface flow, leaching, uptake by crops). The P legacy in soils is associated with widespread eutrophication and soil degradation in many ecosystems.

P fertilizers are derived from mined rock-phosphate which is a rapidly diminishing and finite resource (Gilbert, 2009). At current rates of extraction, global commercial phosphate reserves will be depleted in 50-100 years (Sattari et al., 2012). Further, remaining reserves will decrease in quality and increase in cost (Cordell et al., 2009). Additionally, the increasing demand for agriculture commodities has put pressure on producing higher yields increasing the global demand and prices of P fertilizers (Chowdhury et al., 2017). Therefore, the search for new alternatives to P fertilizers is a real concern.

The P legacy stocks in soil can play a vital role as a reservoir of P if is properly exploited. Some studies show that accumulated P in some agricultural soils could be sufficient to sustain maximum crop yields worldwide for about 100 years if it were fully available for plants (Khan et al., 2007). Practices such as the use of phosphorus solubilizing bacteria (PSB) and organic acids have intended to accelerate soil P transformation to plant-available forms, however these practices are not yet well spread or easily scalable and probably not effective in many contexts.

Phosphorus in plants

Plants have different P requirements depending on crop species and developmental stage (Veneklaas et al., 2012). For instance, the P requirement for optimal growth is in the range of 3 to 5 mg g^{-1} dry weight (DW) during the vegetative stage of growth (Lambers et al., 2011). However, plants that have evolved on severely P-impoverished soils contain an order of magnitude less P in their leaves (Lambers et al., 2011). In contrast, P toxicity is observed at concentrations higher than 10 mg g^{-1} DW. Despite this fact, P toxicity in plants is rare because plants down-regulate their Pi transporters involved in net P uptake when supplied with more P than required (Dong et al., 1998). Interestingly, the total P in top soils (0-15 cm) typically ranges from 50 to 3000 mg kg^{-1} depending on soil parent material, soil texture, vegetation coverage and soil management history (Sanyal and De Datta, 1991). Currently, P fertilization inputs into arable lands double the actual plant uptake capacity. For instance, a global annual application assessment showed that input of P fertilizer and manure for the period 1965-2007 in North America was 500 kg ha^{-1} and cumulative P uptake was approximately 250 kg ha^{-1}. Thus, only half of the applied P was up taken by harvested crops (Sattari et al., 2012). Therefore, there is a

need to understand the impact of P legacy on microbial communities associated to the plant rhizosphere.

Impact of synthetic fertilizer and plant domestication on soil beneficial microbes

Domestication of plant species has substantially contributed to human civilization, but also caused a strong decrease in the genetic diversity of the most common crop cultivars. This overall decrease in diversity may have affected the ability of plants to establish beneficial associations with rhizosphere microbes (Perez-Jaramillo et al., 2016). The transition from wild species to modern agricultural varieties has centered on selecting traits for high yields and within a context of increasing levels of nutrient inputs and relatively high nutrient availability. There is evidence to suggest that during this transition root morphology, anatomy and physiological processes such as plant-root microbial association in soils were affected by the substantial genetic shift and constant exposure to fertilizers (Schmidt et al., 2016).

The green revolution dramatically boosted food production through the breeding of high-yielding crop varieties and the use of fertilizers, pesticides and excess water. More than 50 years later, the environmental consequences are becoming apparent. Some of these impacts are polluted waterways, eightfold increase in N-based fertilizer and a decrease in the reserves of phosphorus rock globally. There is an increasing concern that agricultural intensification leads to large-scale ecosystem degradation and loss of productivity in the long-term (Hartmann et al., 2015). Some of the implications include soil degradation by accumulation of fertilizers (Tilman et al., 2002) as well as the loss of symbiotic relationships between soil microbes and plant roots (Perez-Jaramillo et al., 2018). The effectiveness of plant root and microbial synergies is influenced by management practices impacting soil biological communities (Junaidi et al., 2018). It has been shown that applications of herbicides, pesticides and tillage can lead to a shift of the

microbial community composition and function (Sessitsch et al, 2005; Lo, 2010; Garbeva et. al 2008).

The rhizosphere microbiome of wild versus cultivated plants

A meta-analysis by Perez-Jaramillo et al (2018) investigated how plant domestication affected the composition of the root-associated microbiome in different plant species such as Arabidopsis, sugar beet, barley, and lettuce, and their older relatives. The study showed that domestication led to a consistent change in the abundance of Bacteroidetes, Actinobacteria, and Proteobacteria phyla. Bacteroidetes was consistently enriched in the rhizosphere of wild or older relatives and Proteobacteria and Actinobacteria were consistently enriched on the roots of modern plant types. (Zachow et al., 2014; Bulgarelli et al., 2015; Cardinale et al., 2015). Wild relatives of the common bean (*Phaseolus vulgaris*) nurtured a higher abundance of Bacteroidetes, while the modern bean accessions were dominated by Actinobacteria and Proteobacteria (Perez-Jaramillo et al., 2018). Similarly, Bulgarelli et al. (2015) investigated the structural and functional diversification among communities associated with wild and domesticated accessions of barley (*Hordeum vulgare*). It was found that Flavobacteriaceae family (Bacteroidetes phyla) was significantly present in wild type, but not in domesticated accessions. A different study in non-crop plants (Schlaeppi et al., 2014) used four *Arabidopsis thaliana* relatives and demonstrated that interhost species microbiota was mostly similar, despite their phylogenetic distance (~13 million years). This study also revealed a largely conserved and taxonomically narrow root microbiota comprised of members from Actinomycetales, Burkholderiales, and Flavobacteriales (Schlaeppi et al., 2014).

Other studies suggest that domestication has altered plant-roots and beneficial relationships in mycorrhizal symbiosis, rhizobia and other microbes. For instance, wild ancestors

and landraces of wheat, breadfruit and maize benefited more from mycorrhizal symbiosis than modern cultivars (Hetrick et al., 1992; Xing et al., 2012; Sangabriel-Conde et al., 2014). In a legume-rhizobia symbiosis study, Kiers et al. (2017) showed that newer soybean cultivars had less ability to reach their full symbiotic potential in the presence of mixed rhizobial strains compared to older cultivars.

The goal of this thesis

The goal of this thesis is to contribute to our limited understanding of the impact of P in the plant rhizosphere microbiome of crops, which is necessary to provide evidence on the effects of fertilization on soil health, and therefore plant productivity. In addition, it is of interest to understand the potential beneficial microbial taxonomy linked to wild relatives, which holds a huge potential to elucidate beneficial interactions between plants and microbes.

The first research question aims to determine if the application of excessive P has a detrimental effect on the soil bacterial communities as well as in predictive microbial enzyme activity. Blueberry is a recently domesticated crop and until 1911 it was harvested entirely from the wild. Blueberries are sensitive to fertilization hinting to close relationships with beneficial microbes related to soil nutrition. Therefore, blueberry provides a valuable plant model to understand how P fertilization affects the composition of the rhizosphere microbiome. Studying bacterial communities in two closely related blueberry cultivars with a short history of domestication can evidence interesting microbial changes in responses to P supplementation. A second research question aimed to decipher if the changes in microbial community composition in response to P amendment (found in the blueberry study) are characteristic of either wild or domesticated rhizosphere microbiomes within the *Solanum* genus, shared among the 6 potato genotypes studied. To answer this question, a comparative root microbiome study was

performed, characterizing the bacteria communities in the rhizosphere of modern potato plants and their progenitors and assessing the change in relative abundance of the most abundant microbial taxa present under P amendment. At present, no research has been done on potato plants and their relatives to assess P fertilizer effects on rhizosphere bacteria community composition.

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CHAPTER 2: PHOSPHORUS ADDITION SHIFTS THE MICROBIAL COMMUNITY IN THE RHIZOSPHERE OF BLUEBERRY (*Vaccinium corymbosum* L.)[1]

Introduction

Phosphorous (P) fertilization is a major restricting factor for crop production in many soils due to its low chemical mobility and bioavailability (Sharma et al., 2013, Hinsinger, 2001). Generally, only between 10% to 20% of inorganic P fertilizer applied in the first year is up taken by crops. Inherent soil properties and climate limit its availability; while the majority of the applied P accumulates in the soil as residual P (Syers et al., 2008). Residual P may be utilized by crops for many years P (Syers et al., 2008, Nuruzzaman et al., 2005) depending on soil P sorption capacity, soil pH and crop species (Sanchez, 1977).

In addition, P is naturally present in most soils in insoluble organic forms, derived from plant residues and soil microorganisms, and it has to be mineralized to inorganic phosphate to be fully available as a nutrient (Turner et al., 2005). Some plants have developed specialized mechanisms to increase P absorption from soils by secreting phosphatases that catalyze the hydrolysis of ester–phosphate bonds to soluble orthophosphate (Miller et al., 2001). In addition, many plants have adaptive strategies such as root growth and rhizosphere processes to cope with limited P availability (Lyu et al., 2016, Lambers et al., 2006, Shen et al., 2011). For example, P-deficient *Brassica napus* has high P influx rates (P absorption per unit of root) (Marschner et al.,

[1] Hugo A. Pantigoso, Daniel K. Manter and Jorge M. Vivanco (2018) *Rhizosphere*, 7, pp. 1-7
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2007), whereas P-deficient *Triticum aestivum* has high root/shoot ratios to enhance P-acquisition efficiency under P-deficient conditions (Lyu et al., 2016, Wang et al., 2008).

Among the rhizosphere processes, some plants have developed co-evolutionary interactions with rhizosphere microbes to solubilize P from soil. A considerable number of microbial species show P solubilization capacity and these include bacteria: *Pseudomonas* and *Bacillus* (Illmer and Schinner, 1992), actinomycetes: *Streptomyces* and *Micromonospora* actinomycetes (Hamdali et al., 2008), cyanobacteria: Anabena sp. and *Scytonema* sp. (Sharma et al., 2013); and fungi: *Aspergillus* and *Penicillium*. Phosphate Solubilizing Microorganisms (PSM) facilitate P uptake by plant roots through various mechanisms including: (1) release of complexing or mineral dissolving compounds e.g. organic acid anions, siderophores, protons, hydroxyl ions or CO_2; (2) liberation of extracellular enzymes (biochemical P mineralization); and (3) the release of P during biological P mineralization (Sharma et al., 2013). In spite of these attributes of PSM, shifts in root morphology and rhizosphere processes that allow more effective foraging and nutrient uptake in lower synthetic input environments might have been lost in commercial agriculture compared to natural ecosystems (Schmidt et al., 2017). Domestication of plant species may have negatively affected the ability of plants to establish beneficial associations with rhizosphere microbes such as those that solubilize P (Pérez–Jaramillo et al., 2015, Wissuwa et al., 2009, Bulgarelli et al., 2013). For instance, Szoboszlai et al. (2015) found a structurally and functionally different rhizosphere microbial community in Balsas teosinte (*Zea mays* subsp. *parviglumis*) compared to domesticated cultivars of sweet corn and popping corn.

Blueberry is a recently domesticated crop and until 1911 it was harvested entirely from the wild (Wilson, 1908). The first commercial varieties were introduced in the US market in

1916 and it is nowadays cultivated worldwide (Lobos and Hancock, 2015). Blueberries have a rich interaction with the soil microbial community. A recent microbiome study in wild blueberry shows that the composition of associated bacterial communities differ between managed and natural wild blueberry habitats, confirming that agricultural management can affect rhizosphere microbiomes (Yurgel et al., 2017). Blueberries have developed a strong interaction with ericoid mycorrhizae for the uptake of nitrogen and phosphorus in soils (Jeliazkova and Percival, 2002), and are very sensitive to fertilization most likely due to the fact that it has recently been domesticated for commercial agriculture. Proper nutrition is particularly important for this crop, for instance high P levels may cause deficiencies of other elements, such as Zn, Cu, Fe and others (Bingham, 1963). Therefore, highbush blueberry provides a perfect plant model to understand how P fertilization affects the composition of the rhizosphere microbiome and plant health.

Here, two commercial blueberry cultivars were used to understand the effect of increasing P fertilization on the rhizosphere microbiome of this new crop. It was determined that the microbiome was influenced by low vs. high levels of P amendment to the soil. Those microbiome patterns were correlated with the predicted activity of enzymes related to P solubilization.

Methods and materials

Plant material, fertilizer characteristics and growth conditions

This experiment was conducted at the Horticulture Research Center of the Colorado State University. Southern high-bush blueberry cultivars ‘Misty’ (low chill) and ‘Biloxi’ (no chill) were obtained from a commercial nursery (Fall Creek Nursery Inc, Lowell, OR). On November 16, 2016, 6-weeks old blueberries were transplanted to 3.8 l pots containing a mix of

1:2 pine forest soil (Grey Rock forest, Poudre Canyon, Bellvue, CO)
and sphagnum peat (Canadian Sphagnum Peat Moss, Sun-Grow, Hubbard, OR). Super Triple Phosphate (0–45-0) was applied in 4 rates equivalent to 0, 50, 101 and 192 kg ha^{-1}. The P applied was estimated assuming a lowest phosphorus content in soil. The conversion of fertilizer rates per pot was determined assuming a plow layer of soil weighs 1569 t per hectare. Plants were irrigated three times a week to container capacity with sprayers. The average temperature of the greenhouse was 20 °C to 26 °C during the 12 weeks the experiment last. Treatments were arranged in a randomized complete block design with 5 replicates per treatment. A research randomizer software version 4.0 of open access was used for this purpose (http://www.randomizer.org). Every repetition consisted of a single plant per pot.

Soil and plant sampling

Rhizosphere soil from each plant was collected at the end of the experiment by gently removing the plants from the pots, shaking the plants, and obtaining the soil attached to the roots (~30 gr per plant). A total of 40 rhizosphere soil samples were pooled into bags as individual samples and then stored at 2 °C. Plant samples were immediately processed, shoot and root fresh weights were recorded. Dry biomass was estimated in the laboratory after oven drying.

Nutrient analysis

Leaves were collected at the end of the experiment and oven-dried at 80 °C for 2 days. For soil, 50 g of bulk soil per repetition and per every treatment were collected and stored at 2 °C. After biomass data was recorded, 5 samples of 30 g of leaves were obtained from each repetition per every treatment. Leaves and soil samples were sent to the Soil, Water and Plant Testing Laboratory to determine Nitrogen, Phosphorus and Potassium content in leaves and soils. pH for every soil treatment was also recorded.

Soil microbial DNA extraction

Total DNA was extracted from each rhizosphere sample (n = 20) using a DNeasy Power Soil® DNA isolation kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA was quantified using a Spectrophotometer (Thermo Scientific NanoDrop 2000c, Vernon Hills, IL); all isolated DNA had an absorbance ratio (A260/A280) between 1.8 and 2.0.

Quantitative PCR: 16S analysis

Quantitative PCR (qPCR) amplification of the bacterial 16 S ribosomal RNA (rRNA) genes (V1–V3 hypervariable region) was performed with the 27 F and 388 R primers (Lane et al., 1985, Marchesi et al., 1998). The reaction mix of 20 µL contained 2.0 µL of each primer (10 µM), 4 µL HPLC water and 10 µL Maxima SYBRgreen 2× (cat # K0242, Thermo-Fisher Scientific). The DNA samples were diluted to a concentration of 3 ng μL^{-1}, 2 uL was used per reaction. Amplification was performed as follows: 1) 95 °C for 5 min, 2) 95 °C × 40 s, 55 °C × 120 s, 72 °C × 60 s, repeated 30 times, 3) 72 °C × 7 min. Genomic DNA isolated from *Pseudomonas putida* KT2440 was used as an external standard in order to calculate 16S rRNA copies per g soil FW extracted assuming a *P. putida* genome size of 3.174 fg and seven 16S rRNA copies per genome. qPCR efficiency was 90% and could detect as little as 100 *P. putida* genomes in a single PCR reaction.

Sequencing libraries were constructed by amplification of the V4 region of the 16S rRNA gene using primers 515F and 806R following the protocol for the Earth Microbiome Project (Gilbert et al., 2014). Briefly, amplicon libraries containing Illumina adaptors and 12 bp Golay barcodes were generated for each sample, cleaned using AmPure beads, quantified with

PicoGreen, and pooled in equimolar ratios prior to sequencing at the University of Illinois Genomics Core facility using a 2× 250 MiSeq flow cell (Illumina, San Diego, CA). Paired-end sequence reads were concatenated and all combined 16S sequences were filtered, trimmed and processed with the DADA2 (R bioconductor package), (Callahan et al., 2016) implementation included in the open source bioinformatics tool myPhyloDB version 1.2.1 (Manter et al., 2016). Briefly, all primers were removed from each sequence using the open source Python program Cutadapt (Martin, 2011) and sequence variants were inferred using the default pipeline in DADA2. Each sequence variant identified in DADA2 was classified to the closest reference sequence contained in the Green Genes reference database (Vers. 13_5_99) using the usearch_global option (minimum identity of 97%) contained in the open source program VSEARCH (Rognes et al., 2016).

The abundance of phosphorous cycle genes, E3.1.3.2*, appA, phoA, phoD, phoN, pqqC* (see Table 1 for more details) in each 16S library was predicted using myPhyloDB's implementation of PICRUSt (Langille et al. 2013). Briefly, myPhyloDB reports the proportion of the microbial community that maps to the gene of interest, i.e., proportion of the community with the specified gene. Total gene-specific abundance (copies g^{-1} soil FW) was calculated as the product of the proportion of the community with the specified gene and the total 16S copies (copies g^{-1} soil FW) determine from the initial sample qPCR.

Table 1. KEGG orthologues selected for PICRUSt analysis.

	KEGG		
Process	Gene	Entry	Definition
Decomposition	E3.1.3.2	K01078	acid phosphatase
	appA	K01093	4-phytase / acid phosphatase
	phoN	K09474	acid phosphatase (class A)
	phoA	K01077	alkaline phosphatase
	phoD	K01113	alkaline phosphatase D
Solubility	*pqqC*	K06137	Pyrroloquinoline-quinone synthase

Statistical analysis

Significant differences between phosphorous fertilization rates on plant biomass was analyzed by one-way ANOVA. Phosphorous related genes (see Table 1) were analyzed by two-way ANOVA. For each gene, the proportion of the community with the specified gene was determined by phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) based on the predicted taxonomy of each representative 16 S rRNA sequence (Langille et al. 2013).

The effect of P rate and varietal differences in bacterial community composition (16S rRNA sequences mapped to Green Genes database (Vers. 13_5_99) was visualized by constrained Principal Coordinates Analysis (capscale in R vegan package).
Bacteria phyla with different total abundances (16S copies g^{-1} soil) at the low (0 or 50 kg ha^{-1}) or high (101 or 192 kg ha^{-1}) were tested by differential expression analysis based on negative binomial distributions using the edgeR package (Robinson et al., 2010). Differential expression analysis of total abundances between low and high P rates was also determined for all OTU (99% genetic distance) that mapped to the selected P cycle genes (see above).

Results

Biomass analysis

The study was conducted using two different varieties of blueberries. The effect of P amendments was apparent in both varieties; however, 'Biloxi' exhibited more vigorous growth compared to 'Misty'.

As expected, the dry biomass increased significantly with the application of 50 and 101 kg ha^{-1} of phosphorous compared to the untreated control; this was observed in both

varieties. In addition, the 192 kg ha^{-1} treatment did not differ in biomass dry weight from the 101 kg ha^{-1} treatment for 'Biloxi' (Fig. 1A) and 'Misty' (Fig. 1B).

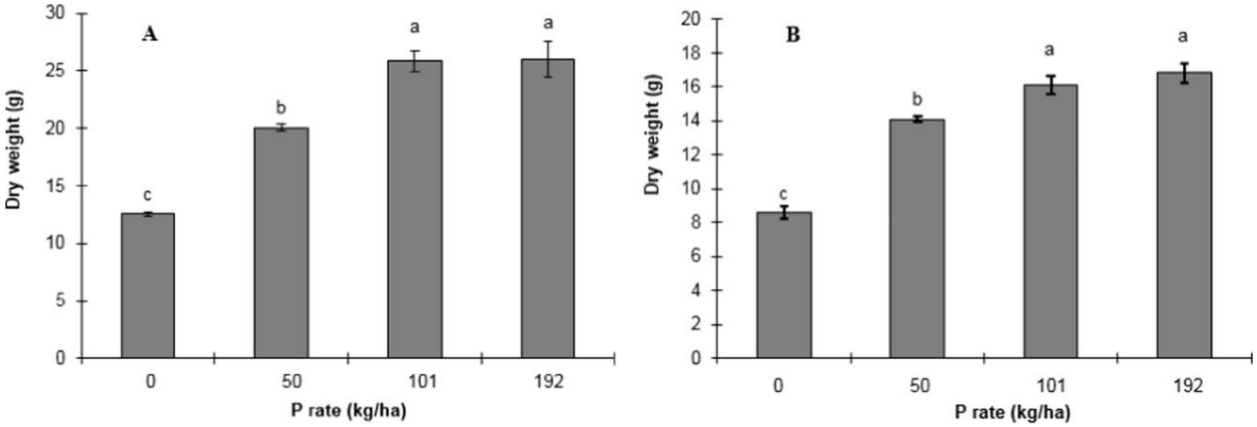

Figure 1. (**A)** Dry biomass analysis of 'Biloxi' cultivar treated with increasing P levels. Graph shows increment in weight (g) when higher P levels were supplied. (**B)** Dry biomass analysis of 'Misty' cultivar treated with increasing P levels. After 101 kg ha^{-1} plants stopped growing in response to P supply.

Soil and leaf phosphorus analysis

Soil and leaf samples corresponding to the treatments were analyzed. The data shows that the content of extractable P in soils gradually augmented from 47.6 mg kg^{-1} to 601 mg kg^{-1} in the Biloxi variety following the increasing amendments of P in the soil. For Misty, the same pattern was observed with values ranging from 43.2 to 716 mg kg^{-1}. The pH values for both varieties decreased with P application rates going from 4.4 (0 kg kg^{-1} P) to 3.7 (191 kg kg^{-1}). In the P leaf analysis, the treatments follow the same pattern of P content in soil. P content in leaves accumulated from 738 mg kg^{-1} to 2774 mg kg^{-1} for Biloxi (Fig. 2A), and from 606 to 1726 mg kg^{-1} for Misty (Fig. 2B).

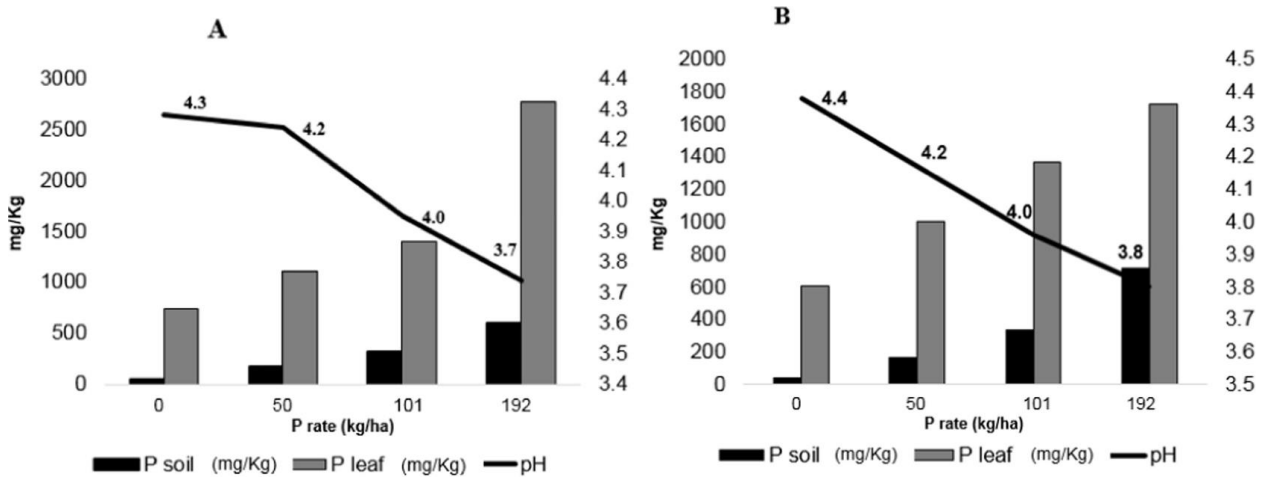

Fig. 2. (**A**). Available P analysis in soil and leaf (mg kg^{-1}) for Biloxi. P content in soil and leaves incremented with increasing P amendments. (**B).** Available P analysis in soil and leaf (mg kg^{-1}) for Misty. pH values decrease with increasing P available levels.

Effect of P amendment on blueberry soil microbial composition

The effect of P amendments on blueberry soil microbial community was determined by Illumina sequencing analysis. The Principal Coordinates Analysis (PCoA) explains 29.1% of the total variation in the data. Also, PERMANOVA analysis identified significant variation in the soil microbial community for the interaction between varieties and P fertilization (P = 0.001). Bray-Curtis distances between samples using PCoA showed dissimilarities in the microbial communities at the blueberry variety level (P=0.001). Furthermore, the 101 and 192 kg ha^{-1} treatments differentiated statistically (P=0.001) in microbial composition from the 0 and 50 kg ha^{-1} treatments. These data clearly show a switch in soil microbial community composition between the low and high P treatments (Fig. 3).

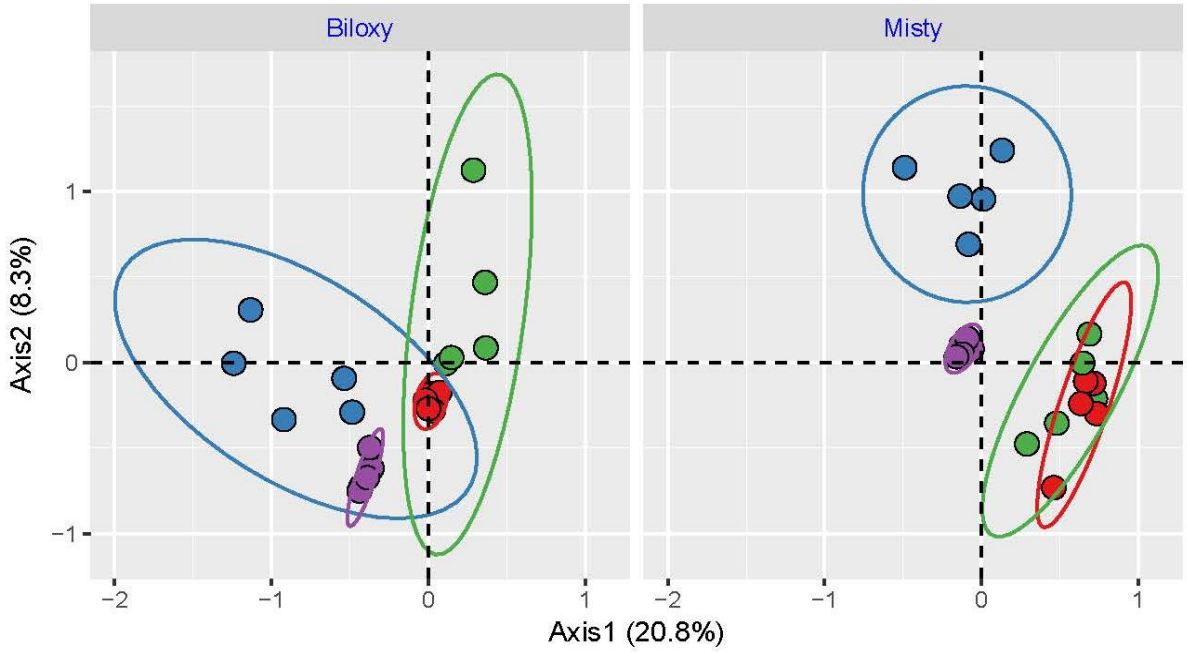

Fig. 3. Principal Coordinate Analysis (PCoA) showing soil microbiome sequencing data from Biloxi and Misty under increasing levels of P fertilizer. Symbols: red – 0 kg ha^{-1} P; green – 50 kg ha^{-1} P; purple – 101 kg ha^{-1} P; blue – 192 kg ha^{-1} P (For interpretation of the references to color in this figure, the reader is referred to the web version of this article).

Subsequently, a deeper analysis to determine the influence of P application on blueberry rhizosphere soil microbial community composition was conducted. The analysis determined the abundance of specific phyla levels present when either P content was low or high. Of the 10 most abundant phyla, six phyla (Proteobacteria, Acidobacteria, Verrucomicrobia, Planctomycetes, Gemmatimonadetes, and OD1) were more abundant under low P rates; and four phyla (Actinobacteria, WPS-2, Bacteroidetes, and TM7) more abundant under high P rates (Table 2).

Table 2. Differential abundance (16S copies g^{-1} soil) table for bacterial phyla at low (0 or 50 kg ha^{-1}) and high (101 or 191 kg ha^{-1}) P rates for both blueberry varieties, 'Misty' and 'Biloxi'.

Means					Likelihood		Discovery	
Overall	High P	Low P	logFC	logCPM	Ratio	p-value	Rate	Phylum
7292.3	8162.8	6421.9	-0.346	18.2	18.7	0.000	0.000	Actinobacteria
6656.2	6369.2	6943.2	0.124	18.1	4.7	0.030	0.054	Proteobacteria
3999.4	3637.7	4361.0	0.262	17.4	19.8	0.000	0.000	Acidobacteria
2167.0	1992.1	2341.9	0.233	16.5	5.8	0.016	0.036	Verrucomicrobia
760.9	699.0	822.8	0.235	15.0	7.8	0.005	0.016	Planctomycetes
643.9	756.8	531.0	-0.511	14.7	22.4	0.000	0.000	WPS-2
494.4	441.9	546.9	0.307	14.4	5.4	0.020	0.040	Bacteroidetes
326.2	379.6	272.7	-0.477	13.8	12.8	0.000	0.002	TM7
297.9	258.3	337.5	0.386	13.6	6.9	0.009	0.022	Gemmatimonadetes
186.5	155.6	217.5	0.483	13.0	3.7	0.053	0.092	OD1
125.8	178.4	73.1	-1.285	12.4	16.4	0.000	0.000	Cyanobacteria
93.0	75.3	110.7	0.556	12.0	5.6	0.018	0.036	Armatimonadetes
92.4	98.2	86.6	-0.181	12.0	0.5	0.474	0.626	FCPU426
50.3	40.8	59.7	0.547	11.1	3.0	0.083	0.137	FBP
37.1	28.5	45.8	0.680	10.7	1.7	0.198	0.297	Chloroflexi
35.8	23.1	48.4	1.062	10.7	7.8	0.005	0.016	Elusimicrobia
33.8	30.3	37.2	0.291	10.6	0.4	0.548	0.646	AD3
26.6	26.5	26.7	0.011	10.2	0.0	0.978	0.998	Chlamydiae
18.1	20.9	15.3	-0.444	9.7	0.4	0.515	0.646	Firmicutes
12.3	4.6	20.1	2.095	9.3	12.8	0.000	0.002	Chlorobi
6.8	7.5	6.0	-0.300	8.5	0.2	0.668	0.735	TM6
6.5	6.5	6.5	0.002	8.5	0.0	0.998	0.998	Spirochaetes
2.2	0.3	4.1	3.149	7.5	7.2	0.007	0.021	BHI80-139
1.3	0.3	2.2	2.441	7.1	1.4	0.232	0.319	Lentisphaerae
0.5	0.7	0.3	-0.808	6.7	0.4	0.548	0.646	OP3
0.5	0.0	1.0	3.108	6.7	7.8	0.005	0.016	GN02
0.5	0.0	0.9	3.046	6.7	7.8	0.005	0.016	Fibrobacteres
0.3	0.3	0.3	-0.016	6.6	0.0	0.993	0.998	Nitrospirae
0.1	0.1	0.0	-1.017	6.5	2.1	0.146	0.229	OP11

Effect of P rate on acid phosphatases activity

The group of acid phosphatases were mapped in all varieties and treatments. The acid phosphatases (APases) analyzed were E3.1.3.2, appA and phoN. Comparisons were conducted between the low (0 and 50 kg ha^{-1}) and high (101 and 192 kg ha^{-1}) P rates among the two blueberry varieties. It was observed that when either variety was treated with increasing

concentrations of P, it caused progressively lower relative abundance of all three APases (Figs. 4A, 4B, 4C). ‘Misty’ showed an overall higher relative abundance compared to Biloxi for the three APases. However, there was a bigger change in relative abundance from low to high P levels for Biloxi. APase E3.1.3.2 was the acid phosphatase most abundant in the rhizosphere samples compared to the other two APases assessed.

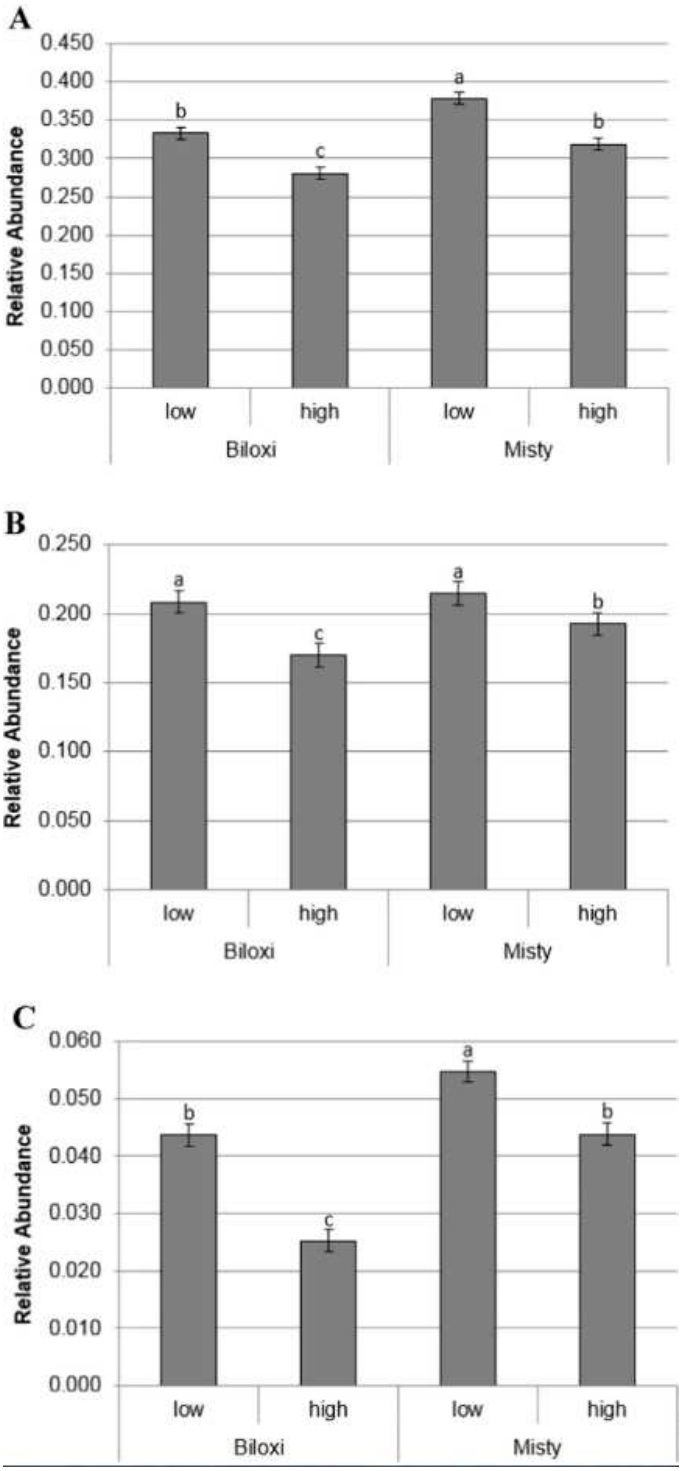

Figure 4. (**A).** Expression analysis of acid phosphatase E3.1.3.2. 'Misty' and 'Biloxi' had a very similar variation in relative abundance (copies g^{-1} soil FW) of E3.1.2.2; 15.8% and 15.96%, respectively when phosphorus rates increased from low to high levels. (**B).** Expression analysis

of acid phosphatase appA. ‘Misty’ and ‘Biloxi’ had a very similar variation in relative abundance of appA; 22.7% and 10.8% respectively when phosphorus rates increased from low to high levels. (**C).** Expression analysis of acid phosphatase phoN. ‘Misty’ and ‘Biloxi’ had a very similar variation in relative abundance of phoN; 48.9% and 12.7% respectively when phosphorus rates increased from low to high levels.

Effect of P rate on alkaline phosphatases activity

The alkaline phosphatases analyzed were phoD and phoA. Comparisons were conducted between the low (0 and 50 kg ha^{-1}) and high (101 and 192 kg ha^{-1}) P rates in the two blueberry varieties. It was observed that when either variety was treated with increasing concentrations of P, it caused no statistically significant difference in relative abundance for phoD alkaline phosphatase (Fig. 5A). For phoA, the same pattern was shown only for ‘Misty’ (Fig. 5A). Soil pH conditions were acidic during the entire experiment, pH varying from 3.8 to 4.5 units (Figs. 2A, 2B); this may explain the overall small variation of relative abundance of alkaline phosphatases compared to acidic phosphatases.

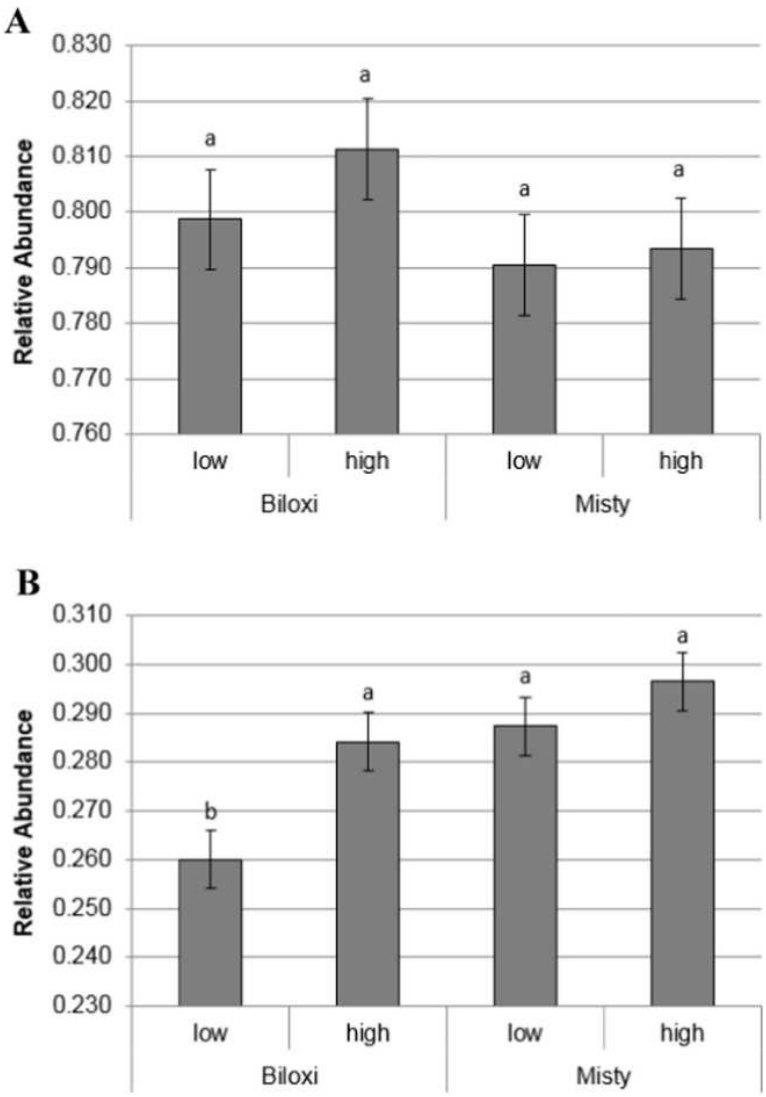

Figure 5. (**A).** Expression analysis of alkaline phosphatase phoD. 'Misty' and 'Biloxi' did not have significant variation in relative abundance of phoD; 1.6% and 0.4% respectively when phosphorus rates increased from low to high levels. (**B).** Expression analysis of alkaline phosphatase phoA. 'Misty' and 'Biloxi' had a variation in relative abundance of phoA; of 9.3% and 3.2% respectively when phosphorus rates increased from low to high levels.

Effect of P rate on Pyrroloquinoline – quinone Synthase (PQQ) activity

Pyrroloquinoline - quinone Synthase (PQQ) is an enzyme related to phosphorus solubility. PQQ enzyme was mapped and analyzed in all varieties and treatments. Comparisons were conducted between the low (0 and 50 kg ha^{-1}) and high (101 and 192 kg ha^{-1}) P rates among the two blueberry varieties. It was observed that when either variety was treated with increasing concentrations of P, it caused progressively lower relative abundance of PQQ (Fig. 6). The overall relative abundance for 'Misty' and 'Biloxi' was very similar.

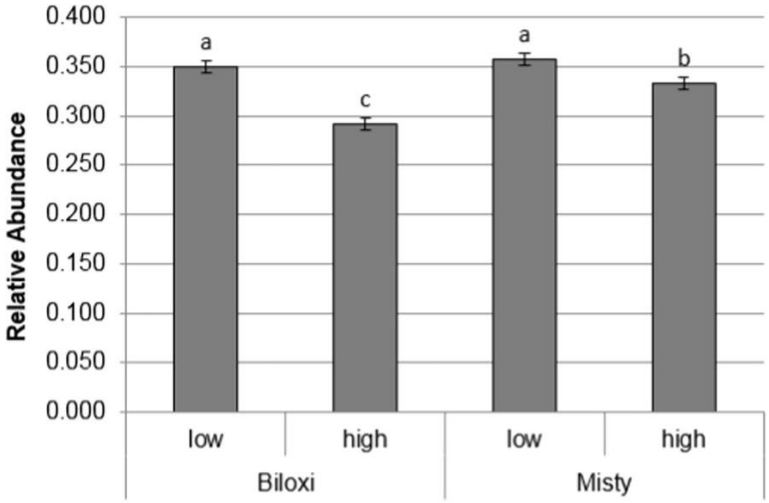

Figure 6. Expression analysis of Pyrroloquinoline-quinone synthase PQQ. 'Misty' and 'Biloxi' had a very similar variation in relative abundance of PQQ; 16.6% and 6.8% respectively when phosphorus levels increased from low to high levels.

Discussion

This study aimed to explore the effect of P fertilization amendments on soil bacterial community composition in two varieties of commercial blueberry (*Vaccinium* sp. var. "Misty" and "Biloxi"). Phosphorus is a frequent nutrient applied to blueberries for successful commercial growth and production. Nutrient requirements in perennial fruit crops such as blueberry depend

on new biomass production in vegetative and reproductive tissues (Bryla et al., 2012). Our data showed increased dry biomass for the highest P treatments (101 kg ha^{-1} and 192 kg ha^{-1} P) for both blueberry varieties. It was found that biomass increased with each subsequent P level (up to 101 kg ha^{-1}); whereas, at the highest P level (192 kg ha^{-1}) biomass did not increase. These results suggest that the needs of P for the plant were satisfied near 101 kg ha^{-1}.

Soil microbial communities are frequently susceptible to nutrient inputs (Leff et al., 2015). For instance, N fertilization typically reduces microbial biomass, respiration rates (Ramirez et al., 2012, Ramirez et al., 2010b) and specific functional groups of microbes, including ammonia oxidizers and mycorrhizal fungi (Wessén et al., 2010). In the case of P amendments there is substantially less information available. Impact of phosphorus on microbial composition has been mainly investigated in grasslands, pastures or forest (Leff et al., 2015, Adair et al., 2013, Tan et al., 2013, Robbins et al., 2018, Liu et al., 2012). For instance, Tan et al. 2013 showed that after decades of phosphate application the diversity and abundance of the bacterial community increased in grazed pasture. Taxonomic composition was also reported where the relative abundance of Acidobacteria was higher in the soils not amended with phosphorus; but Bacteroidetes and Actinobacteriaincreased gradually in soils amended with P. In contrast, Robbins et al. 2018 evaluated bacterial community response in soils from a long term phosphorus fertilization trial. They found that differences in soil P contribute only 3.4% of the variation in soil bacterial communities, suggesting that the soil biome is largely resilient to long-term P fertilization.

In our study, the microbial community detected in the rhizosphere of the two blueberry cultivars Misty and Biloxi was comprised of 29 bacterial phyla with the four most abundant phyla (Actinobacteria – 31%; Proteobacteria – 29%, Acidobacteria – 17%,

and Verrucomicrobia – 9%) comprising nearly 90% of the community. This composition resembles the microbial composition reported by Yurgel et al. 2017 in natural and managed blueberry habits. In our study, of the 10 most abundant bacteria phyla, six phyla (Proteobacteria, Acidobacteria, Verrucomicrobia, Planctomycetes, Gemmatimonadetes, and OD1) were more abundant under low P rates; and four phyla (Actinobacteria, WPS-2, Bacteroidetes, and TM7) more abundant under high P rates. These results suggest that the bacterial community taxa present in low P conditions may contain organisms that make insoluble phosphorus available for the roots. For instance, phosphorus solubilizing bacteria have been reported within different orders of Proteobacteria taxa as Enterobacteriales, Lactobacilalles among other groups (Mohammad Bagher et al., 2015). In contrast, higher concentration of P in soil causes a greater uptake of P by some microbes (Chauhan et al., 1981) as inorganic P is immobilized into organic P forms (Büneman et al., 2012). In our study under high soluble P rates fast growing bacteria such as Actinobacteria and Bacteroidetes were abundant presumably competing with roots in P acquisition.

Modern cultivar selection has normally been executed under high fertility soil conditions with a particular focus on yield (Wissuwa et al., 2009). It is possible that crop selection undermined the capacity of some crops to access soil nutrients existing in forms not readily available for the plant (Wissuwa et al., 2009, Drinkwater and Snapp, 2007). For instance, studies comparing mycorrhizal competence of several wheat cultivars found that varieties developed prior to 1950 were more reliant on mycorrhizal symbiosis than modern wheat cultivars (Hansen et al., 2017, Hetrick et al., 1992, Hetrick et al., 1993). Thus, the roots of traditional varieties are more efficient in up taking P than modern wheat cultivars (Batten, 1992, Wissuwa and Ae, 2001). In this study, a shift in microbial community composition in

abundance and diversity in response to P amendment is reported. Similar to plant biomass, the soil microbiome of blueberry was influenced by P level and appeared to differentiate based on low P (0 and 50 kg ha^{-1}) and high P (101 and 192 kg ha^{-1}) rates. Based on this evidence, results suggest that when P is supplied to the plants over a particular threshold level the root-microbial associations that favor acquisition of phosphorus from the soil are disrupted.

Wild and recently domesticated blueberries are grown under low nutrient availability, acidic and infertile soils to which they are well-adapted. These conditions resemble forest systems where they are native (Wolf et al., 1987). In acidic soils, P solubilization appears often to be associated with lower pH (Hall and Okali, 1978) resulting in increased P uptake by plant roots (Kucey et al., 1989). In our study, the microbial acid phosphatases (E3.1.3.2, appA and phoN) showed a consistent decline in its activity from low to high P rates. Similarly, PQQ phosphatase decrease its activity when exposed to higher P rates. Contrasting, due to acidic conditions of the soil tested, the alkaline phosphatases, phoD remained without a significant change in its abundance. These enzymatic patterns are consistent with other studies that show that low P levels in the soil induce soil microbial phosphatase synthesis (Yun and Kaeppler, 2001). It is also important to mention that Misty showed slightly higher relative abundance than Biloxi in five phosphatases. Four of those phosphatases were related to phosphorous decomposition (E3.1.3.2; *appA; phoN; phoA*) and the last one was involved in phosphorous solubility (*pqqC*). In contrast, Biloxi showed higher plant biomass compared to Misty. It could be hypothesized that Biloxi has a higher efficiency to convert mineral phosphorous in biomass, and accordingly higher yield (Hummer et al., 2007), compared to Misty. However, Misty might strongly rely on microbial phosphatases to uptake P from soil.

Conclusions

Data presented herein indicate a response of microbial composition to higher P applications. Additionally, the diminished activity of acid phosphatases at high phosphorus rates suggests that the oversupply of P reduces the ability of the plant to sustain beneficial relationships with P solubilizing bacteria. Future studies should focus on how crop domestication has affected root traits and rhizosphere microbial interactions in the soil.

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CHAPTER 3: EFFECTS OF PHOSPHORUS FERTILIZATION ON THE RHIZOSPHERE BACTERIAL COMMUNITY OF CULTIVATED AND NON-CULTIVATED POTATOES

Introduction

The microbial community associated with the rhizosphere can have a profound effect on nutrition, growth and health of plants in agro-ecosystems (Chaparro et al., 2012). Root symbionts and free-living microbes are responsible for the acquisition of nutrients that are otherwise inaccessible to plants (van der Heijden et al., 2007). The effectiveness of plant root and microbial synergies are influenced by management practices impacting soil biological communities (Junaidi et al., 2018). It has been shown that applications of herbicides, pesticides and tillage can lead to a shift of the microbial community composition and function (Sessitsch et al., 2005; Lo, 2010; Garbeva et al., 2008). However, our understanding of the effects of excessive fertilization, phosphorus in particular, and its impact in rhizosphere microbiome and crop health remain sparse.

Phosphorus (P) is an essential element in plant nutrition and a major limiting nutrient in agriculture (Syers et al., 2008). After being applied to soils, P can be fixed by soil sorption capacity or precipitated by free aluminum (Al^{+3}), iron (Fe^{+3}) or calcium (Ca^{+3}) depending on soil pH, thus becoming unavailable to plants (Havlin et al., 1999). Additionally, the P uptake in most crops is generally restricted to 10-20% of the P applied (Wolf et al., 1987); thus, farmers are continually encouraged to increase P fertilization. The oversupply tends to accumulate as P residual in soils (Tilman et al., 2001). This residual P fertilizer is also known as ‘P legacy’ and is defined as the difference of cumulative inputs including mineral P fertilizer, atmospheric

deposition and weathering and cumulative outputs, such as P export crops, erosion, leaching and run off (Zhu et al., 2018). Currently, P fertilization inputs into arable lands double the actual plant uptake capacity. For instance, input of P fertilizer and manure for the period 1965-2007 in North America was 500 kg ha^{-1}, while cumulative P uptake was approximately 250 kg ha^{-1}; thus, only half of the applied P was utilized by crops (Sattari et al., 2011). Thus, there is a need to understand the impact of P legacy in microbial communities associated to plant rhizospheres.

Cultivated potatoes (Solanum tuberosum L.) were domesticated between 8000 to 10000 years ago from wild *Solanum* species native to the Andes of southern Peru (Hardigan et al., 2017). In its natural habitat wild potato species grow in nutrient limited (and P deficient) soils; suggesting that there is a rich interaction between plants and beneficial microbes to support adequate plant nutrition. Over the last 150 years, potatoes have been bred mainly for below ground traits, such as tuber development to increase yield (Hawkes, 1997; Friedman, 2006). In conjunction, many cultivars have been bred within a context of frequent P fertilization during domestication. As a consequence, high fertility conditions used during plant breeding and selection may have hampered the ability of plants to initiate beneficial interactions with microbes related to nutrient solubilization. (Perez-Jaramillo et al., 2015; Wissuwa et al., 2008). In natural environments numerous microorganisms including species of fungi and bacteria are effective at releasing P from organic and inorganic soil P pools through mineralization and solubilization, respectively (Bhattacharyya and Jha, 2012). Short and long-term studies have shown that this symbiosis can be altered when high levels of P are added to soils. A short- term greenhouse experiment in blueberries showed that high levels of P (192 kg ha^{-1}) reduced the abundance of genes associated with phosphatases production in soil, accompanied by a shift of the microbial community associated to the rhizosphere (Pantigoso et al., 2018). In a different study,

incremental addition of soil phosphate in a long-term P fertilization study significantly affected arbuscular mycorrhizal fungal community composition (Williams et al., 2017). Furthermore, this microbial shift caused by fertilization over time seems to be more prevalent in modern crop varieties. For example, the nitrogen-fixing endophyte *Azoarcus spp.* preferentially colonized wild rice species and old varieties than modern cultivars (Engelhard et al., 2000). A more recent study demonstrated that an indigenous landrace of maize grown using little or no fertilizer showed high levels of nitrogen (N) fixation, deriving up to 82% of the plant N from the atmosphere. This was mainly due to diazotrophic bacteria which were not present in modern maize varieties (Van Deynze et al, 2018).

To successfully manage beneficial interactions between plant roots and microbes we need to better understand the impacts of soil management on root associated microbial communities during the domestication process. In this study, it is hypothesized that P would not only affect biomass response and P use efficiency but shift microbial community composition in cultivated and non-cultivated potato genotypes. It was anticipated a higher dissimilarity in microbial taxa for high P treatments in non-cultivated vs. cultivated potatoes, and a very similar microbial composition among all 6 genotypes not subjected to P amendments.

Methods and materials

Selection of potato accessions and growth conditions

This study was performed in a greenhouse at the Plant Growth Facility (PGF) of the Colorado State University (CSU), Fort Collins, CO, United States between March and May 2018. Three commercial cultivars of *Solanum tuberosum* widely grown in North America were selected: ‘Yukon Gold’, ‘Russet Burbank’ and ‘Red Norland’; one direct progenitor of the three

commercial cultivars: *Solanum tuberosum* subsp. *tuberosum* (PI 595492); one landrace: *Solanum tuberosum* subsp. *andigena* (PI258907) and one wild type*: Solanum bulbocastanum* (PI 275184). These three cultivated and three non-cultivated potato species were chosen to evaluate the impact of P fertilization on soil microbial communities in a crop domestication gradient. Certified organic tubers from commercial cultivars were obtained from Grand Teton Organics farm, Idaho, U.S. Tubers were pre-sprouted during ten days at room temperature. Botanical seeds from the three non-cultivated accessions were obtained from the Potato Gene Bank, Sturgeon Bay, Wisconsin, U.S. Potato seeds were pre-treated with gibberellic acid at 2000 ppm and surface disinfected with 3% of sodium hypochlorite and left for 24 hours in distillate water. Seed were pre-germinated in wet filter paper and moved to Hoagland nutritional solution (4.33 g L^{-1}) for nine days. Small plants were transplanted to a substrate made of vermiculite, sand and soil (1:1:1) during ten days for fast growth. Tubers of the three cultivars and seedlings of three accessions were transplanted to 15 cm pots containing a mix of two parts sand and one-part soil collected (0-20 cm depth) from a field previously planted with cover crop (field 7 South) from the Agricultural Research, Development and Educational Center – South farm (ARDEC) of Colorado State University. This soil was collected on March 13, 2018, soil texture is Sandy Clay Loam, pH of 8.4 and organic matter of 3.0 %. Plants were grown on benches in the greenhouse for approximately 60 days.

Fertilizer super triple phosphate (0-45-0) was applied in 3 rates equivalent to 0, 67 and 133 kg ha^{-1}. The three P applications (each application time used the set ratio beforehand mentioned) were conducted every 15 days to all six different potato genotypes. Plants were irrigated to field capacity with sprayers. The range of average min and max temperatures in the greenhouse during the experiment was 16 °C to 30 °C. Treatments were arranged in a

randomized complete block design with 10 replicates per treatment. Every replicate consisted of a single potato plant per pot. A research randomizer software version 4.0 of open access was used for this purpose (http://www.randomizer.org).

Rhizosphere soil collection and plant biomass sampling

Rhizosphere soil from each plant was collected at the end of the experiment by gently removing the plants from the pots, shaking the whole plant to separate the bulk soil exposing the plant roots. The remaining soil still attached to the root hairs (rhizosphere soil) was carefully removed with the hands and then pooled into bags as individual samples and then stored at 2 °C. Fresh plants, shoots and roots, were weighed and fresh biomass recorded immediately after harvest. Plants were then placed in an oven for 4 days at 80 °C, and weighed. Plants propagated from tuber seeds ('Russet Burbank', 'Red Norland' and 'Yukon Gold') started with approximately ~3 g of dry weight over the other potato genotypes (*Solanum tuberosum* subsp. *tuberosum*, *Solanum tuberosum* subsp. *andigena* and *Solanum bulbocastanum*), for this reason a correction was made and a reduction of 3 g to the average dry weight in potato cultivars was considered and shown in Fig 7A.

Plant nutrient analysis

Dried plant samples from each treatment and repetition (n=10) were mix and homogenized and sent to the Soil, Water and Plant Testing Laboratory at CSU to determine total P content. One sample per each treatment was sent to the lab for analysis.

DNA extraction, PCR, and 16S rRNA amplicon sequencing

Total DNA was extracted from each rhizosphere sample using a DNeasy Power Soil DNA isolation kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. The

DNA was quantified using a spectrophotometer (Thermo Scientific NanoDrop 2000c, Vernon Hills, IL). All isolated DNA had an absorbance ratio (A206/A280) between 1.8 and 2.0.

Quantitative PCR (qPCR) amplification of the bacterial 16 S ribosomal RNA (rRNA) genes (V1–V3 hypervariable region) was performed with the 27 F and 388 R primers (Lane et al., 1985, Marchesi et al., 1998). The reaction mix of 20 µL contained 2.0 µL of each primer (10 µM), 4 µL HPLC water and 10 µL Maxima SYBRgreen 2× (cat # K0242, Thermo-Fisher Scientific). The DNA samples were diluted to a concentration of 3 ng μL^{-1}, 2 uL was used per reaction. Amplification was performed as follows: 1) 95 °C for 5 min, 2) 95 °C × 40 s, 55 °C × 120 s, 72 °C × 60 s, repeated 30 times, 3) 72 °C × 7 min. Genomic DNA isolated from Pseudomonas putida KT2440 was used as an external standard in order to calculate 16S rRNA copies per g soil FW extracted assuming a P. putida genome size of 3.174 fg and seven 16S rRNA copies per genome. qPCR efficiency was 90% and could detect as little as 100 P. putida genomes in a single PCR reaction.

Sequencing libraries were constructed by amplification of the V4 region of the 16S rRNA gene using primers 515F and 806R following the protocol for the Earth Microbiome Project (Gilbert et al., 2014). Briefly, amplicon libraries containing Illumina adaptors and 12 bp Golay barcodes were generated for each sample, cleaned using AmPure beads, quantified with PicoGreen, and pooled in equimolar ratios prior to sequencing at the University of Illinois Genomics Core facility using a 2× 250 MiSeq flow cell (Illumina, San Diego, CA). Paired-end sequence reads were concatenated and all combined 16S sequences were filtered, trimmed and processed with the DADA2 (R bioconductor package), (Callahan et al., 2016) implementation included in the open source bioinformatics tool myPhyloDB version 1.2.1 (Manter et al., 2016). All primers were removed from each sequence using the open source

Python program Cutadapt (Martin, 2011) and sequence variants were inferred using the default pipeline in DADA2. Each sequence variant identified in DADA2 was classified to the closest reference sequence contained in the Green Genes reference database (Vers. 13_5_99) using the usearch_global option (minimum identity of 97%) contained in the open source program VSEARCH (Rognes et al., 2016).

Statistical analysis

Significant differences between P fertilization rates on plant biomass per genotype was analyzed by one-way ANOVA with a significance of $P<0.05$. Phosphorus content in plant was determined using only one repetition, therefore not statistical analysis is presented.

The effect of P rate and genotype differences in bacterial community composition (16S rRNA sequences mapped to Green Genes database (Ver. 13_5_99) was visualized by constrained Principal Coordinates Analysis (PCoA) (capscale in R vegan package).

Bacteria phyla with different relative abundances (16S copies g^{-1} soil) at the low (0 kg ha^{-1}) or high (133 kg ha^{-1}) were tested by ANcOVA for the top abundant taxa across six potato genotypes.

Results

Above and Below Plant biomass analysis

The effect of P amendments was apparent in all six genotypes. However, tuber-bearing genotypes ('Russet Burbank', 'Yukon Gold', 'Red Norland' and *Solanum tuberosum* subsp. *tuberosum*) in the presence of P fertilizer exhibited more vigorous growth compared to non-tuber bearing species (*Solanum bulbocastanum* and *Solanum tuberosum* subsp. *andigena*) after correction (Figs 7A, B). *Solanum tuberosum* subsp. *tuberosum*, a direct progenitor of modern potatoes (including the three commercial cultivars of this study), showed significantly less biomass compared to the other tuber-bearing cultivars. Above ground dry biomass increased significantly ($p=0.05$) with the application of 67 kg and 133 kg ha^{-1} of P compared to the untreated control (0 kg ha^{-1}) for all genotypes, except for *Solanum bulbocastanum* (Figs 1A, B). Belowground dry biomass significantly increased for cultivar 'Yukon Gold' and the progenitor *Solanum tuberosum* subsp. *tuberosum* when exposed to phosphorus ($p=0.05$; Fig. 1B). Tuber-bearing genotypes showed a higher biomass in below ground organs (roots and tubers), contrary to what was observed in non-tuber bearing species, which concentrated most of their biomass in their above ground organs (leaves and stems). Potato genotypes responded in differently in biomass allocation to the same P level addition. For instance, 'Russet Burbank' outperformed other tuber-bearing potatoes in above biomass and 'Yukon Gold' excelled in below ground biomass. For non-tuber bearing, *Solanum tuberosum* subsp. *andigena* accumulated more above ground biomass than *Solanum bulbocastanum* and the opposite occurred for below ground biomass.

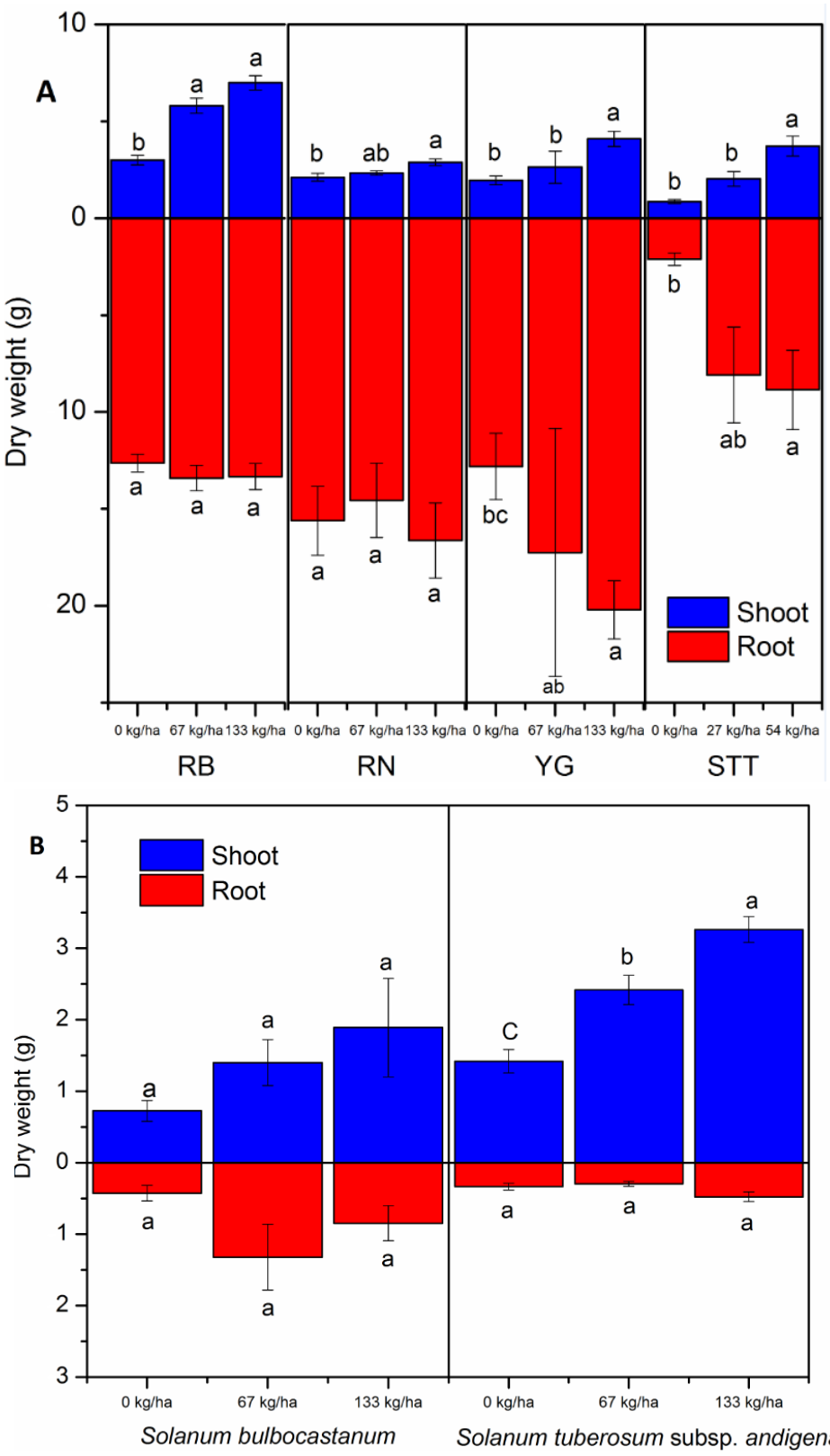

Figure 7. Dry biomass of six potato genotypes treated with 0, 67 and 133 kg ha^{-1} of P. **(A)** Above (leaves and stems) and below (roots and tubers) ground biomass of four tuber-bearing

accessions. From left to right: 'Russet Burbank' (RB), 'Red Norland' (RN), 'Yukon Gold' (YG) and *Solanum tuberosum* subsp. *tuberosum* (STT). **(B)** Above and below ground biomass of two non-tuber bearing accessions: *Solanum bulbocastanum* and *Solanum tuberosum* subsp. *andigena*.

Leaf phosphorus analysis

Leaf samples corresponding to each P level (0, 67, 133 kg ha^{-1}) from the 6 genotypes were analyzed (Fig 8). Total P concentration gradually increased for tuber-bearing cultivars and non-tuber bearing types. For P concentration in above ground tissue, non-tuber bearing potatoes had a higher content of P per unit of biomass (mg kg^{-1}) in response to increasing P addition. Further, *Solanum bulbocastanum* doubled the P concentration of tuber-bearing potatoes and *Solanum tuberosum* subsp. *andigena* performed better than all three cultivars. The phosphorus content in shoots for tuber-bearing types ranged from 1040 to 6066 (mg kg^{-1}) while the non-tuber bearing potatoes ranged from 1964 to 8767 (mg kg^{-1}) (Table 1).

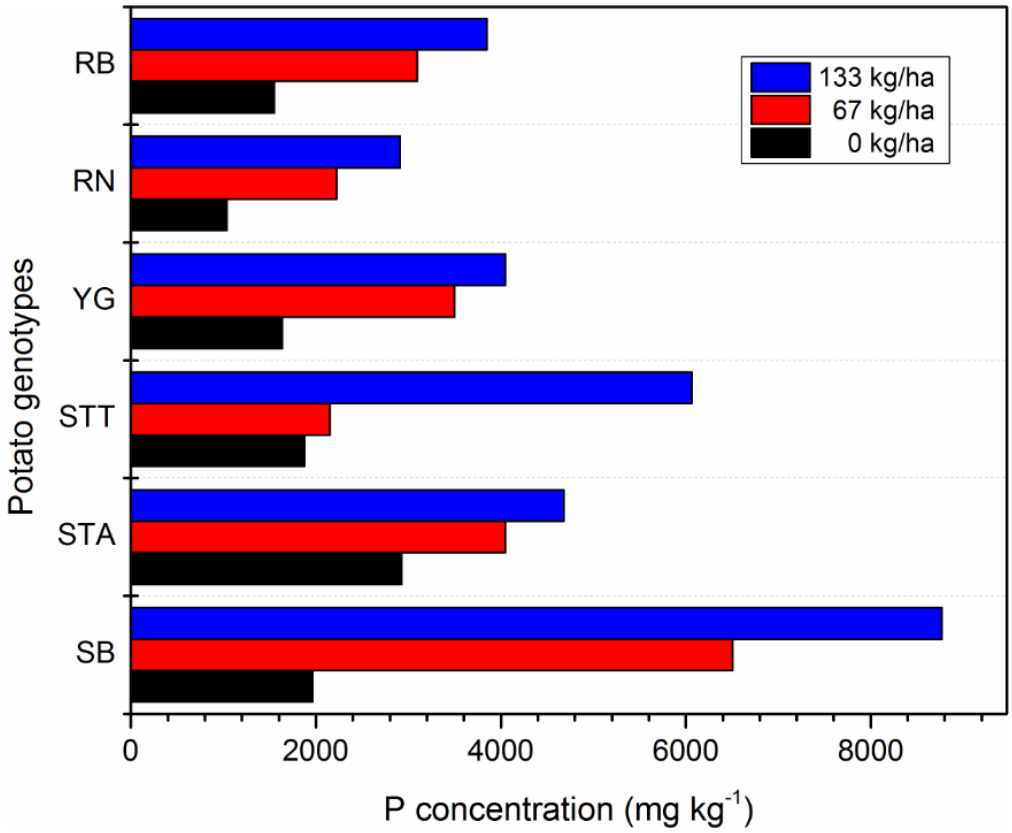

Figure 8. P concentration (mg kg^{-1}) in dry leaves of six potato accessions. *Solanum bulbocastanum* (SB), *Solanum tuberosum* subsp. *andigena* (STA), *Solanum tuberosum* subsp. *tuberosum* (STT), 'Yukon Gold' (YG), 'Red Norland' (RN), 'Russet Burbank' (RB). Each potato accession present three P treatments (0, 67, 133 kg ha^{-1}).

Table 3. P concentration (mg kg^{-1}), dry weight (g) and P concentration (mg g^{-1} DW) in dried leaves of six potato genotypes. P concentrations.

P level	Potato accessions	P concentration (mg kg-1)	Dry weight shoot (g)	Total P(mg g−1 DW)
0 kg/ha	Russet Burbank	1552	3.00	5
27 kg/ha	Russet Burbank	3098	5.80	18
54 kg/ha	Russet Burbank	3852	6.99	27
0 kg/ha	Red Norland	1040	2.11	2
27 kg/ha	Red Norland	2227	2.33	5
54 kg/ha	Red Norland	2912	2.91	8
0 kg/ha	Yukon Gold	1637	1.95	3
27 kg/ha	Yukon Gold	3499	2.63	9
54 kg/ha	Yukon Gold	4050	4.10	17
0 kg/ha	*Solanum tuberosum* subsp. *tuberosum*	1880	0.86	2
27 kg/ha	*Solanum tuberosum* subsp. *tuberosum*	2152	2.02	4
54 kg/ha	*Solanum tuberosum* subsp. *tuberosum*	6066	3.72	23
0 kg/ha	*Solanum tuberosum* subsp. *andigena*	2929	1.42	4
27 kg/ha	*Solanum tuberosum* subsp. *andigena*	4049	2.42	10
54 kg/ha	*Solanum tuberosum* subsp. *andigena*	4684	3.26	15
0 kg/ha	*Solanum bulbocastanum*	1964	0.73	1
27 kg/ha	*Solanum bulbocastanum*	6508	1.40	9
54 kg/ha	*Solanum bulbocastanum*	8767	1.89	17

Effect of P amendment on potato soil microbial composition

The effect of P amendment on microbial communities of potato soil was determined by Illumina sequencing analysis. The Principal Coordinates Analysis (PCoA) showed dissimilarities in microbial communities at the potato genotype level, explaining 22.3% of variation in the data. Also, PERMANOVA analysis identified significant variation in the soil microbial communities for the comparison between genotypes (P=0.001), but not for the comparison between P fertilization rates (P=0.025). Furthermore, PCoA summarizing six potato genotypes and three fertilizer levels showed that all three potato cultivars clustered together while the three non-

cultivated potatoes were grouped individually and further apart (Fig. 9). These data show that microbial community composition is similar among cultivars and different between non-cultivated potato types. In addition, plant genotype drives microbial community composition to a greater degree than increasing P levels (Fig 9). Interestingly, when microbial composition of every genotype was analyzed individually the divergence per P fertilizer level became more apparent. However, 'Red Norland' was the only genotype showing significant differences among P levels. All remaining 5 genotypes (tuber-bearing and non-tuber bearing) did not show a significant separation under P. (Supplemental Fig 1, 2).

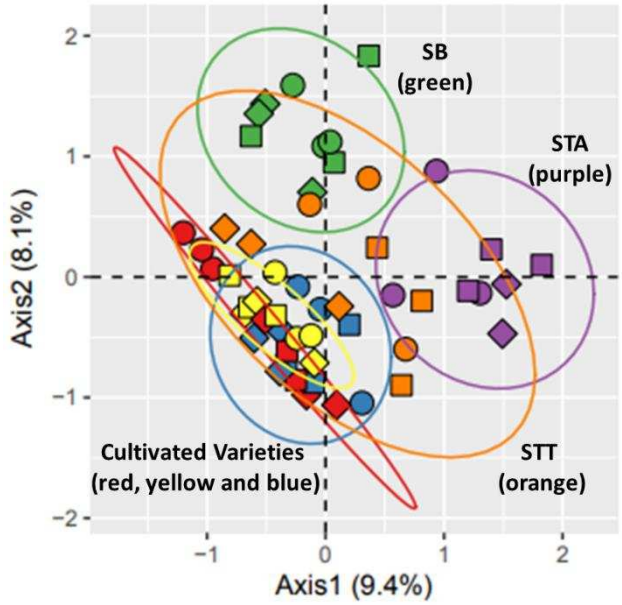

Figure 9. Principal Coordinate Analysis (PCoA) depicting 16S rRNA data from soil samples amended with three levels of P in six potato genotypes. Potato genotype drives the separation of rhizosphere microbial communities. Confidence ellipses are shown around each potato accession: SB: *Solanum bulbocastanum* (green), STA: *Solanum tuberosum* subsp. *andigena* (purple), STT: *Solanum tuberosum* subsp. *tuberosum* (orange), 'Russet Burbank' (blue), 'Red Norland' (red), 'Yukon Gold' (yellow). P levels are: 0 kg ha^{-1} (circle), 67 kg ha^{-1} (square), 133 kg ha^{-1} (Rhomb).

Effect of P on rhizosphere top taxa of tuber-bearing potato types

A deeper analysis was conducted to determine potato rhizosphere taxonomic classification of the top bacteria at families when P content was low (0 kg ha^{-1}) and high (133 kg ha^{-1}). The purpose of this analysis was to identify the change in relative abundance of the most representative families within each potato group (tuber-bearing and non-tuber bearing plants) present in both categories (low and high P) to assess the differential response of increasing phosphorus levels over six potato genotypes following a gradient of domestication. When selecting the 10 most abundant families present in both, low and high P, the number varied for each variety. Some potato genotypes have as low as 6 families (and 4 unclassified) and other up to 9 families (and 1 unclassified).

The tuber-bearing potato group comprised Geodermathophilaceae, Micrococcaceae, Nocardioidaceae, Rhodospirillacea, Sphingomonadaceae, Xanthomonadaceae and unclassified families. These microbial families were consistently present and highly abundant in 'Red Norland', 'Russet Burbank', 'Yukon Gold' and their progenitor *Solanum tuberosum* (Fig. 3). By contrast, families only present in certain potato cultivars but not in others were: Micromonosporaceae ('Red Norland' and 'Yukon Gold'), Flavobacteriaceae ('Russet Burbank' and *Solanum tuberosum* subsp. *tuberosum*), Cystobacteriaceae ('Yukon Gold'), Sinobacteraceae and Nitrospiraceae (*Solanum tuberosum* subsp. *tuberosum*). From these top taxa present across all four cultivars, the family Xanthomonadaceae increased its relative abundance when higher levels of P were supplemented. In contrast, Geodermathophilaceae was the only group that consistently decreased in abundance when subjected to high P, this change was more evident for 'Yukon Gold' and 'Red Norland'. All other microbial families selected in this analysis responded differently to P addition without a noticeable pattern.

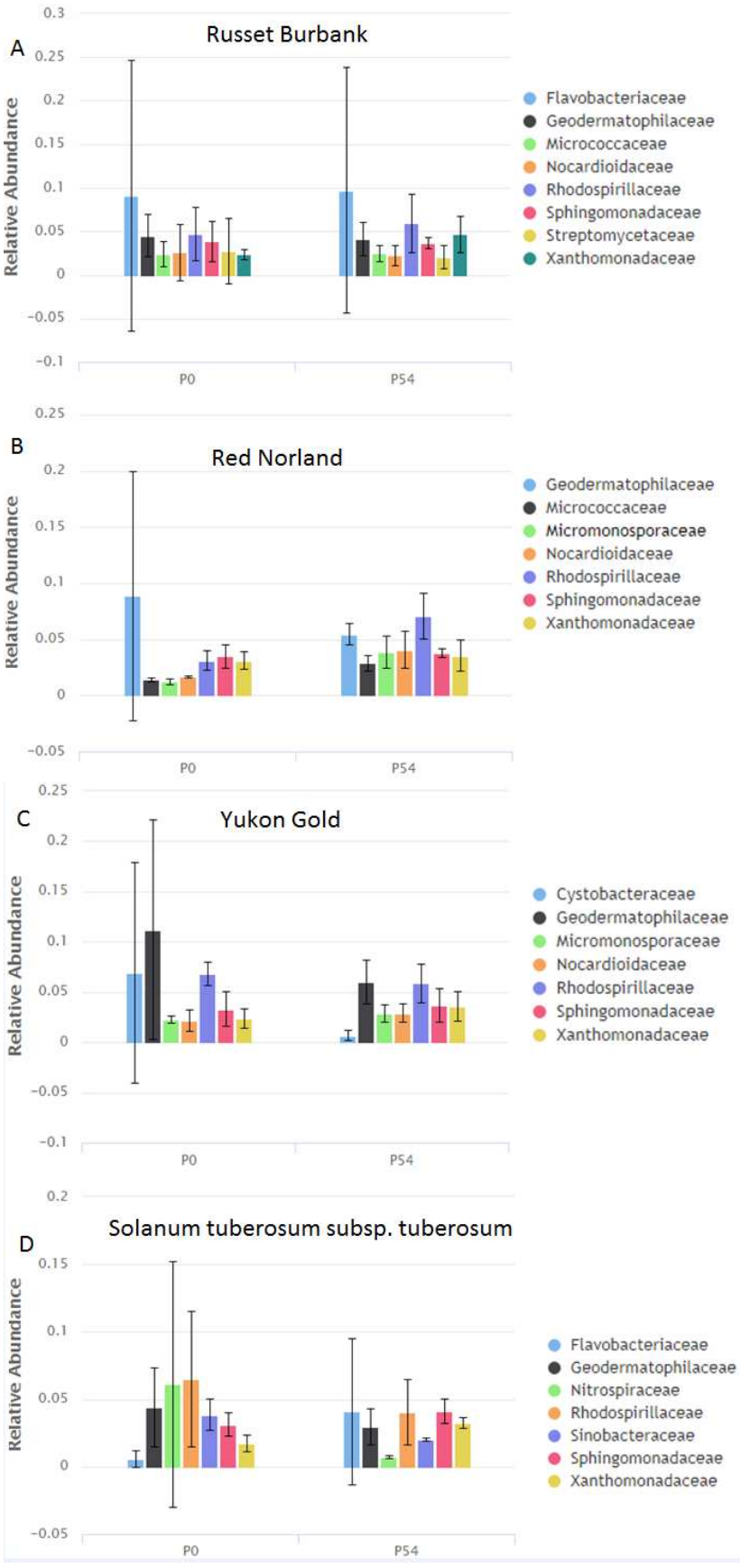
A
Russet Burbank
Relative Abundance
0.3
0.25
0.2
0.15
0.1
0.05
0
−0.05
−0.1
P0
P54
Flavobacteriaceae
Geodermatophilaceae
Micrococcaceae
Nocardioidaceae
Rhodospirillaceae
Sphingomonadaceae
Streptomycetaceae
Xanthomonadaceae
B
Red Norland
Relative Abundance
0.2
0.15
0.1
0.05
0
−0.05
P0
P54
Geodermatophilaceae
Micrococcaceae
Micromonosporaceae
Nocardioidaceae
Rhodospirillaceae
Sphingomonadaceae
Xanthomonadaceae
C
Yukon Gold
Relative Abundance
0.25
0.2
0.15
0.1
0.05
0
−0.05
−0.1
P0
P54
Cystobacteraceae
Geodermatophilaceae
Micromonosporaceae
Nocardioidaceae
Rhodospirillaceae
Sphingomonadaceae
Xanthomonadaceae
D
Solanum tuberosum subsp. tuberosum
Relative Abundance
0.15
0.1
0.05
0
−0.05
P0
P54
Flavobacteriaceae
Geodermatophilaceae
Nitrospiraceae
Rhodospirillaceae
Sinobacteraceae
Sphingomonadaceae
Xanthomonadaceae

Figure 10. Relative abundance of the seven most abundant bacterial taxa at the family level in low (0 kg ha^{-1}) versus high (133 kg ha^{-1}) P levels in non-tuber bearing potatoes: **(A)** 'Russet Burbank', **(B)** 'Red Norland', **(C)** 'Yukon Gold' and **(D)** *Solanum tuberosum* subsp. *tuberosum*.

Effect of P on top taxa in non-tuber bearing potato types

For the non-tuber bearing potatoes Alteromonadaceae, Geodermathophilaceae, Rhodospirillacea, Sphingomonadaceae, Xanthomonadaceae and unclassified were present in both 0 kg ha^{-1} and 133 kg ha^{-1} of P at different relative abundance levels. By contrast, families only present in certain non-tuber bearing cultivars but not in others were: Comamonadaceae (*Solanum bulbocastanum*) and Hyphomicrobiaceae, Nocardioidaceae, Sinobacteraceae, Streptomycetacea and unclassified (*Solanum tuberosum* subsp. *andigena*). The family Alteromonadaceae decreased with incremental phosphorus addition for both species in the group, and Sphingomonadacea increase with high P for *Solanum bulbocastanum* while maintaining same level for *Solanum tuberosum* subsp. *andigena*. All others did not show a positive or negative pattern across genotypes.

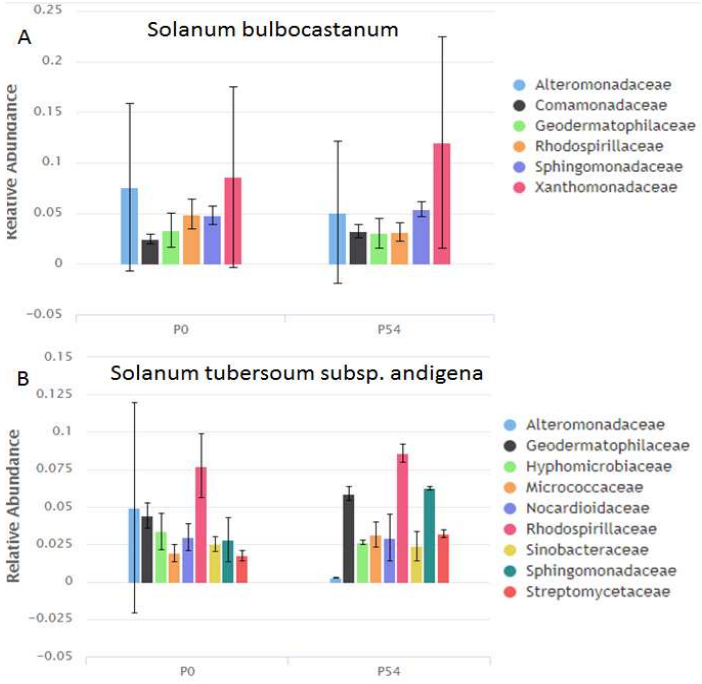

Figure 11. Relative abundance of the top most abundant bacterial taxa at the family level present in low (0 kg ha^{-1}) versus high (133 kg ha^{-1}) P levels in non-cultivated potatoes: **(A)** *Solanum tuberosum* subsp. *tuberosum* and **(B)** *Solanum tuberosum* subsp. *andigena*.

Discussion

From a multitude of biotic and abiotic factors, plant genotype and soil conditions are considered the main drivers of rhizosphere microbial community composition (Berg et al., 2009). In this study, the rhizosphere microbiota of six cultivated and non-cultivated potato plants were analyzed. All accessions were subjected to increasing P addition (0 kg ha^{-1}, 67 kg ha^{-1} and 133 kg ha^{-1}). It was hypothesized that P fertilization would differentially impact the relative abundance of the most abundant taxa in modern varieties (cultivated) vs. wild species/landraces (non-cultivated) of potato plants. Secondly, it was anticipated that changes to rhizosphere microbial communities would be accompanied by changes in biomass and P use efficiency.

Tuber-bearing and non-tuber bearing potatoes responded differently to the level of P addition. Among the tuber-bearing potatoes, some types outperformed others in producing aboveground biomass (i.e. 'Russet Burbank') and other potato types in below ground biomass allocation (e.g. 'Red Norland') indicating that every cultivar has a different nutritional response and efficiency to assimilate P amendments. In contrast, the increment of aboveground biomass in non-tuber bearing types was proportional with the increase on P supply. This may suggest a better response of certain potato types to uptake P from soils. For tuber-bearing potatoes, below ground biomass was considerably higher than above ground biomass. In potato plants up to 40% of the total P may be incorporated in tubers as starch (Hawkesford et al., 2012), these results showed below ground biomass almost tripled the biomass weight for tuber-baring potatoes. Part

of this higher below ground biomass corresponds to the tuber-seed of three cultivars placed at transplanting time; a correction to amend this extra weight was made. Comparatively, above ground biomass of the six potato genotypes responded to P, however only 'Yukon Gold' and *Solanum tuberosum* subsp. *tuberosum* were significantly impacted in belowground organs suggesting a loss in P response for some but not all domesticated potato genotypes. Additionally, P content in above ground tissues showed a higher accumulation of P in non-tuber bearing potatoes. The P content was the highest for *Solanum bulbocastanum* at 133 kg ha^{-1} across all genotypes and P levels. Contrary, the lowest P content score across 6 potato types was found for 'Red Norland' (0 kg ha^{-1}). P content of below ground organs was not determined for this study, however could be necessary to understand total P uptake among potato cultivars.

It was anticipated a higher dissimilarity in microbial taxa for high P treatments in non-cultivated vs. cultivated potatoes, and a very similar microbial composition among all 6 genotypes not subjected to P amendments. It was hypothesized that bacterial composition of all 6 genotypes would remain similar, coming from the same genus *Solanum* sp., allowing us to compare degree of drift of bacterial communities in response to P treatment. At a broader level, our results indicated that the root-associated bacterial community was strongly influenced by the host more so than by the effect of fertilizer addition. This finding supports previous research showing that plants can host their own distinct root microbiota not only at a plant species level, but also at a cultivar level (Schweitzer et al., 2008; Peiffer et al., 2013). However, most of the differences in this study were seen at the species level. Additionally, a shift on bacteria community composition with the addition of P, when individual potato genotypes were analyzed, was observed. This is supported by a recent study showing a shift in microbial community composition with blueberry plants subjected to a high P treatment (0 and 50 kg ha^{-1}) were

different in community similarity compared to a low P treatment (0 and 50 kg ha^{-1} vs 101 and 192 kg ha^{-1}) were amended (Pantigoso et al., 2018). It was observed that incremental P amendments selected certain taxa causing divergence of the whole community in the soil.

A review of the literature shows examples of specific soil microbes affected by short and long-term fertilization. For instance, Williams et al., (2016) demonstrated that barley (*Hordeum vulgare*) can reduce carbon allocation to arbuscular mycorrhizae fungi in response to P fertilization. Similarly, a 22-year study found that elevated N inputs altered the legume rhizobium mutualism (Weese et al., 2015). Recent research showed that the effects of fertilizer on microbes are greater in modern cultivars, presumably due to the unintended impact of plant breeding and high input agricultural practices on co-evolved root-microbiome interactions (Schmidt et al., 2016) and the fact that there is a decoupling between crops and their rhizosphere communities. For instance, mycorrhizal colony responsiveness (increased growth of P uptake of a plant resulting from AMF colonization) to modern wheat cultivars was found to be generally inferior than those in older cultivars. In the same study, P use efficiency was found to be generally higher across wild types (Zhu et al., 2001).

These results show a variety of responses in the relative abundance of the 10 most abundant microbial families within the phyla Proteobacteria, Actinobacteria, Bacteroidetes and Nitrospirae, present in both tuber-bearing and non-tuber bearing potato genotypes. Changes in relative abundance do not necessarily imply a change in absolute number; it represents a shift in the rank order of an organism within a community. This shift could lead to a change in the ability of that organisms to compete for resources and influence ecosystem processes (Goldfarb et al., 2011). Besides effects on microbial biomass and activity, fertilization can influence relative

abundance of different microbial decomposers and solubilizers that display contrasted nutrient requirements (Fanin et al., 2014).

This research found families that remained unchanged (e.g. Micrococcaceae), decreased (e.g. Nitrospiraceae) or increased (e.g. Xanthomonadaceae) in abundance with the addition of P. Alteromonadaceae was the only top abundant family present in non-tuber bearing plants and absent in the tuber-bearing potato group. Further, Alteromonadaceae considerably decreased in abundance with high P levels for both species within the non-tuber bearing group. A deeper analysis at genera level in Alteromonadaceae family showed a drastic decrease of *Cellvibrio sp.* when P increased for both non-tuber bearing potatoes types. *Cellvibrio sp.* is described as biopolymer-degrading bacteria (Roesti et al., 2005). This genus has been found to be an endosymbiont associated with the spores of different arbuscular mycorrhizal fungi (Turrini et al., 2018; Desiró et al., 2014).

Xanthomonadacea family consistently increased in abundance for high P levels in all tuber bearing members and in Solanum bulbocastanum (non-tuber bearing type). Further, *Arenimonas* sp. (a member of the Xanthomonadaceae family) has shown to have catalytic activities as acid and alkaline phosphatases, esterase, lipase, etc. (Makk et al., 2015). *Arenimonas* sp. is also capable of metabolizing casein, gelatin, tyrosine, beta-hydroxybutyric acid, etc. (Chen et al., 2015c). Xanthomonadaceae members are known to obtain carbon (C) from co-occurring microorganisms (Lueders et al., 2006) and thus are important in C and P transformations. *Lysobacter* sp., a genus of the same family, is described as bacterial micro-predators playing an important role in sequestration and turnover of microbial biomass (Lueders et al., 2006). In our studies, *Arenimonas* and *Lysobacter* were found to minimally increase in relative abundance

under high P levels for some potato genotypes (data not shown). Thus, other members of the Xanthomonadacea are induced under high P levels.

In this analysis it was observed that the following families were highly abundant and exclusively present in non-cultivated potato types: Comamonadaceae (*Solanum bulbocastanum*) and Streptomycetacea (*Solanum tuberosum* subsp. andigena). Some members within these families have been reported as P solubilizer bacteria (PSB). For example, *Delfia* sp. a genus member of the Comamonadacea family is able to solubilize tricalcium phosphate in growth media (Chen et al., 2006). Species of the *Streptomyces* sp. genus and members of Streptomycetaceae family are reported to be PSB in wheat (Hamdali et al., 2008a), but also common diseases in potato as *Streptomyces scabiei* (Lerat et al., 2009). In a further analysis, *Streptomyces* sp. was found to increase in abundance under high P for 'Red Norland' and *Delfia* sp. was not present in our soil samples.

For cultivated potato types, some examples of bacteria families containing PSB members are *Chryseobacterium* sp. and *Flavobacterium* sp. genus from Flavobacteriaceae family (Singh et al., 2013; Chen et al., 2006; Pishgar et al., 2019). The latter (*Flavobacterium* sp.) are also responsible of heterotrophic denitrification and associated with plant growth promotion and pathogen protection in the rhizosphere of bell pepper (Kolton et al., 2012). *Micromonospora* sp. from Micromonosporacea family (Hamdali et al., 2008a) are also P solubilizers. A further analysis at genera level found *Flavobacterium* sp. and *Micromonospora* sp. increased in abundance with high P, but we were unable to detect *Chryseobacterium* sp (data not shown). This result suggests that the level of P affects abundance of top taxa differently for most of potato genotypes.

Most of the bacteria previously described in our study belong to Proteobacteria and Actinobacteria phyla. Actinobacteria have been shown to be linked to soil P cycling and a recent study demonstrated that P fertilization modified microbial community structure to a more copiotroph bacterial community (Trabelsi et al., 2017; Fanin et al., 2015). This is supported by the hypothesis that rich-nutrient environments are preferred by bacteria with fast-growing rates as Proteobacteria and Actinobacteria (Fierer et al., 2012). Furthermore, Mander et al. (2012) showed that the frequency of bacteria capable of P solubilization *in vitro* was negatively correlated to soil P levels in three pastures differing in fertilizer management history. In a follow-up study, long-term alteration of soil P status affected the abundance and taxonomic composition of bacteria capable of solubilizing mineral phosphate (Wakelin et al., 2012). These findings show a variety of responses among the top most abundant taxa and sheds light on the main microbial players in P transformation in soils associated with cultivated and non-cultivated potato plants. This study also demonstrated that non-cultivated potatoes are more efficient up taking P compared to their modern relatives. Understanding the compositional changes of bacterial communities in the rhizosphere in response to continuous fertilization can be highly instrumental in plant breeding programs.

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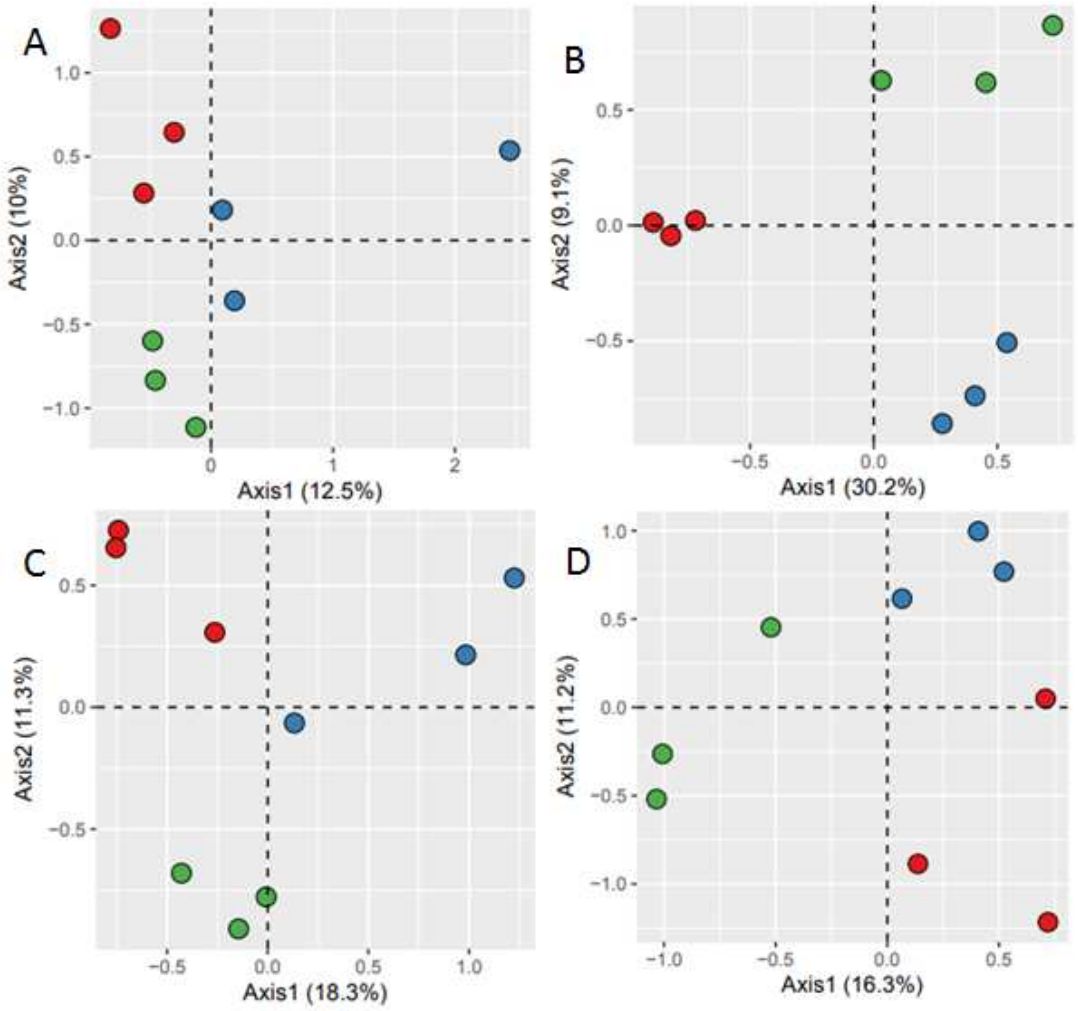

Figure S1. Principal Coordinate Analysis (PCoA) depicting 16S rRNA data from soil samples amended with 3 levels of P in each of the four cultivated potato accessions: (A) 'Russet Burbank' (P=0.71), (B) 'Red Norland' (P=0.029), (C) 'Yukon Gold' (P=0.094) and (D) *Solanum tuberosum* subsp. *tuberosum* (P=0.54). Fertilizer level are colored as follow: 0 kg ha^{-1} (red), 67 kg/ha (blue) and 133 kg ha^{-1} (green).

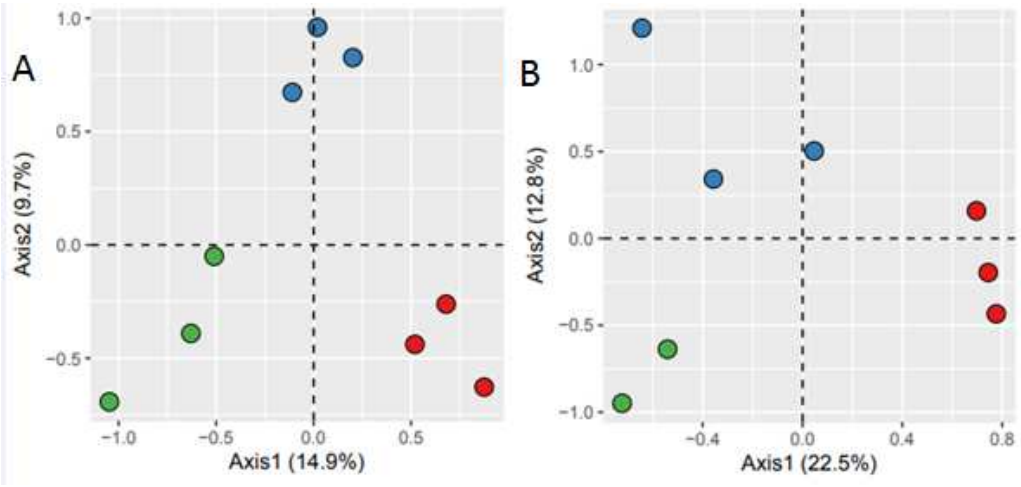

Figure S2. Principal Coordinate Analysis (PCoA) depicting 16S rRNA data from soil samples amended with 3 levels of P in each of the two non-cultivated potato accessions: (A) *Solanum bulbocastanum* (P=0.26) and (B) *Solanum tuberosum* subsp. *andigena* (P=0.06). Fertilizer level are colored as follow: 0 kg ha^{-1} (red), 67 kg ha^{-1} (blue) and 54 kg ha^{-1} (green).