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Soil health and *Verticillium* disease of potato
Part III: Microbial Community Relationships

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Executive Summary:

- Approximately **8,500 bacterial taxa and 10,000 fungal taxa** were identified in potato soils of the PNW
- Both bacterial and fungal communities varied by region, soil order, and texture class, calling for a consideration of these differences in future research
- Soils with a higher microbial biomass were associated with soils with low disease incidence
- Microbial community characteristics correlated with many soil health indicators

Introduction

This is the third and final part in our series reporting about the relationship between soil health and Verticillium wilt. The first two parts emphasized how management (Potato Progress XIX:9) and soil health indicators (Potato Progress XX:11) differed between healthy and diseased fields. This report will examine whether microbial communities of healthy and diseased fields are different and how they are related to soil health indicators.

Verticillium wilt is a common limiting factor of potato production in the Pacific Northwest. The disease can be caused by either *Verticillium dahliae* or *Verticillium albo-atrum*, but the former is prevalent pathogen in the Pacific Northwest (Johnson and Dung 2010). *Verticillium dahliae* has become the predominant pathogen of potatoes because its microsclerotia can survive in soil for over a decade. It is difficult to eradicate from soils using rotation management techniques due to its ability to use numerous weeds as alternative hosts. Conventional disease control has focused on reducing the populations of microsclerotia in soil using broad-spectrum fumigants. But the high costs, potential negative health and environmental concerns associated with the use of fumigants, and future implementation of

stricter regulatory guidelines are strong incentives to find non-chemical control methods for managing this soilborne disease.

An ideal solution to the problem would be if the soil could be made resistant or even better, suppressive to the disease through targeted management practices with the ability to put pressure on the pathogen (Johnson and Dung 2010; Hills et al. 2020). That this is fundamentally possible has been known for some time (Powelson and Rowe 1993). In a benchmark study conducted at Aberdeen, Idaho, Davis et al. (1996) found the effect of certain green manure treatments to be equivalent to that of soil fumigation, with up to 81% reduction in disease severity and a 35% increase in tuber yield. Certain organic amendments may reduce the incidence of verticillium wilt and potato scab, as well as populations of plant pathogenic nematodes to near zero (Conn and Lazarovits 1999). Unfortunately, the disease control efficacy of these treatments was often limited to a specific site and/or product (Lazarovits 2010). Apparently, what works at one site does not necessarily work in another.

What makes a soil suppressive to Verticillium wilt?

The ability of a soil to suppress *Verticillium*, or any other plant pathogen, is primarily through the activities of other microorganisms in soil, although it can be moderated by management, environmental factors, and soil conditions, such as pH, nutrient status, and soil moisture (Fig. 1). Biological suppression is divided into general and specific suppression (Cook and Baker 1983).

General suppression refers to an overall decline in pathogenic microorganisms, most likely from competition for resources with the rest of the microbial community. This resource competition can often be related to the overall abundance and/or richness of the large percentage of microbes that are not pathogenic to plants. Greater numbers make for more competition for carbon and nutrients; greater diversity (numbers of different types of species) is related to the wider use of those resources. Thus, we might predict that soils with more microbial biomass tend to be more suppressive. General suppression can also refer to the alteration of key soil properties, such as pH, that may result from the activity of the microbial community. Many soil health promoting practices, such as use of cover crops, crop rotations, and addition of organic matter to soil, may enhance general suppressiveness (Hills et al. 2020).

Specific suppression means that a particular plant pathogen, such as *Verticillium*, is targeted by a particular organism. Often this is through antagonistic or parasitic/predatory interactions. The production of antibiotics is a classic example of antagonism. A number of microbial taxa (e.g., *Bacillus*, *Pseudomonas*, *Streptomyces*, *Trichoderma*) have been shown to have a biocontrol effect against various pathogens, including *Verticillium* spp. (Berg et al. 2001; Uppal et al. 2008; Wiggins and Kinkel 2005). The desire to introduce a 'silver bullet' biocontrol organism is seductive but often yields inconsistent results (Hills et al. 2020). As with general suppression, modifying agronomic practices may be a more successful strategy to favor the specific suppression of *Verticillium* (Inderbitzin et al. 2018).

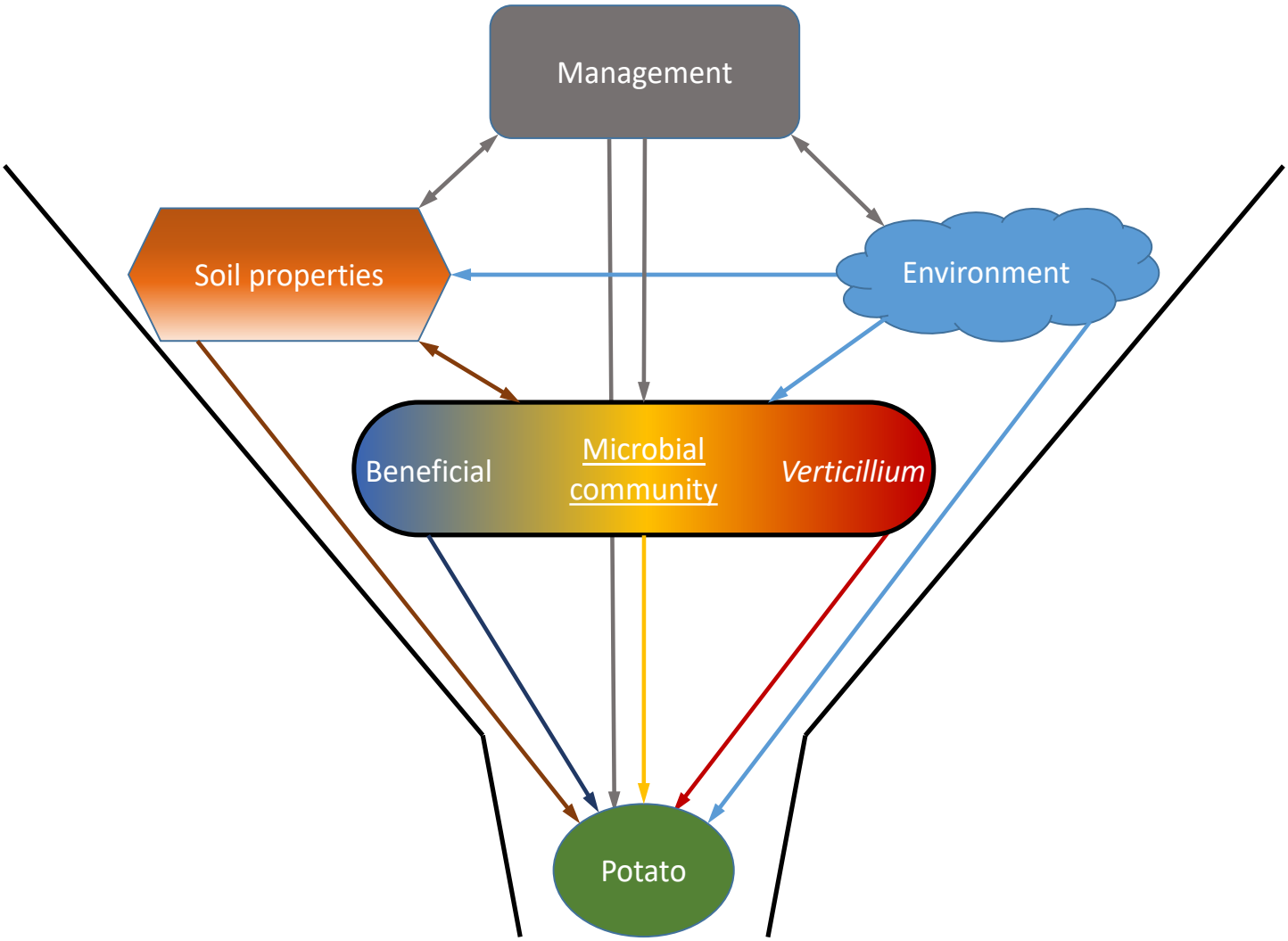

Figure 1. This diagram conveys the major factors contributing the yield and quality of potato production and the central role of the soil microbial community. Management can directly affect potatoes through variety selection and the application of agrochemicals, such as fertilizers and herbicides. It can also have indirect effects through modifying the environment—irrigation as a major modifier, soil properties (e.g., tillage effects on soil structure, addition of organic matter), and the microbial community—most directly through the use of fumigants or possibly through the use of inoculants. The most important environmental factors are precipitation and temperature. These climatic factors affect soil development and thereby soil properties, microbial community structure and activity, potato phenology and growth, and can influence management decisions (e.g., planting time). Soil properties include a number of inherent features, such as texture and mineralogy, which change slowly and are not affected by management, to dynamic soil properties that respond rapidly (weeks to a few years) and are often used as indicators of soil quality (e.g., aggregation, available carbon). Soil properties are important determinants of microbial community structure and activity (e.g., pH, C/N ratio, water content) and directly affect potato growth by providing nutrients and water storage. Indirectly, soil properties influence management decisions (e.g., types and quantities of fertilizer, timing and amount of irrigation). The soil and environmental properties are largely fixed, although some of their properties can be affected by management. Soil microbial communities mediate many of the management, environmental, and soil factors through their role in cycling carbon and nutrients as well as biotic interactions with potatoes. These biotic interactions can be detrimental to potato health (e.g., *Verticillium* wilt) or beneficial (e.g., arbuscular mycorrhizae, nutrient supply). Microbial activity can also influence soil properties such as promoting aggregation, influencing water infiltration (repellency), stabilizing carbon, and altering pH.

Assessing microbial communities

We used two complementary approaches to measure the microbial community: phospholipid fatty acid (PLFA) analysis and DNA sequencing (Fig.2). PLFA analysis measures the lipids in microbial membranes. It provides a measure of microbial biomass based on the total amount of PLFA extracted from a soil sample and uses different types of PLFAs as biomarkers of broad groups of microbes, such as fungi, and Gram-negative and Gram-positive bacteria. From DNA extracted from soil we amplified marker genes for bacteria and archaea (a portion of the 16S ribosomal gene) and fungi (the ITS region of the ribosomal gene). The DNA sequences obtained are useful for identifying the different microbial taxa, providing information about their diversity and composition, and potentially identifying specific taxa associated with susceptibility or suppression.

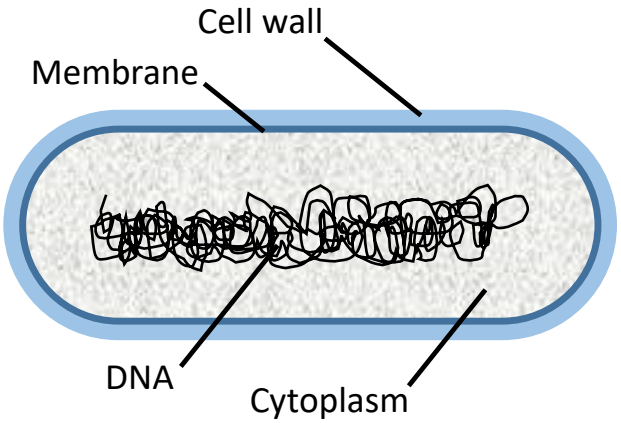

Figure 2. Main components of a bacterium. Phospholipids (PLFAs) are the building materials for cell membranes and vary in their composition between microbial taxa. The DNA molecule is the hard-drive of the cell, storing the information needed to control growth and physiological processes (such as the ability to produce antibiotic chemicals).

Did microbial community data distinguish between healthy and diseased soils?

Total microbial biomass, measured as the sum of all PLFAs, varied more than 10-fold across the 40 sites sampled and was about 33% higher in soils with a low incidence of disease. The higher microbial biomass found in soils selected as having a low incidence of disease nearly met the typical criterion for statistical significance ($p = 0.053$, read: there was a 94.7% probability for biomass being higher in healthy soils). Based on biomarker PLFAs, the biomass of Gram-positive bacteria ($p = 0.022$) and actinomycetes ($p = 0.036$) was greater in soils with a low incidence in disease (Fig. 3); however, there was no statistically significant difference in fungal biomass or the ratio of fungal to bacterial biomass. The differences in Gram-positive and actinomycete biomass were a reflection of differences in total microbial biomass because the relative abundance of these two groups did not differ between soils of low and high disease incidence.

The species richness and diversity of bacteria and fungi were measured from the DNA sequence data, but no differences were found in either

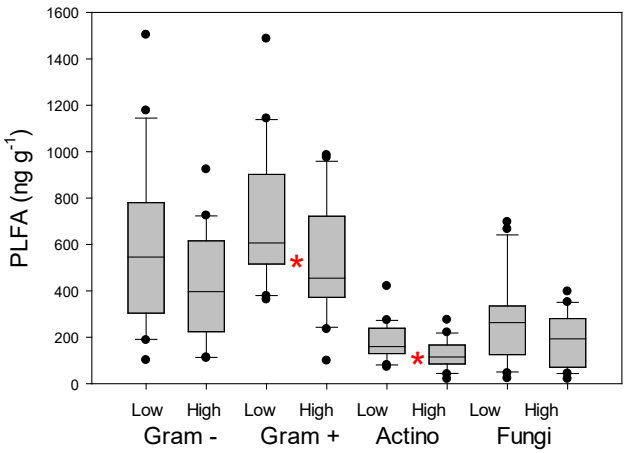

Figure 3. Biomass of bacterial and fungal PLFA biomarkers found in soils categorized as having a low or high incidence of Verticillium wilt. The red "*" indicates a statistically significant difference ($p < 0.05$, $n = 40$) between diseased and healthy soils for Gram-positive bacteria and Actinobacteria.

microbial group in soils of low or high disease incidence (Fig. 4).

The composition, or structure, of the microbial communities was assessed using the relative abundance of PLFAs or of DNA sequences. In examining microbial community structure, we looked for differences not only between soils of low and high disease incidence, but we also examined differences among regions (Columbia River Basin, Klamath Basin, Snake River Plains), soil orders (Aridisols, Entisols, Inceptisols, Mollisols), and CASH texture class (coarse, medium, fine).

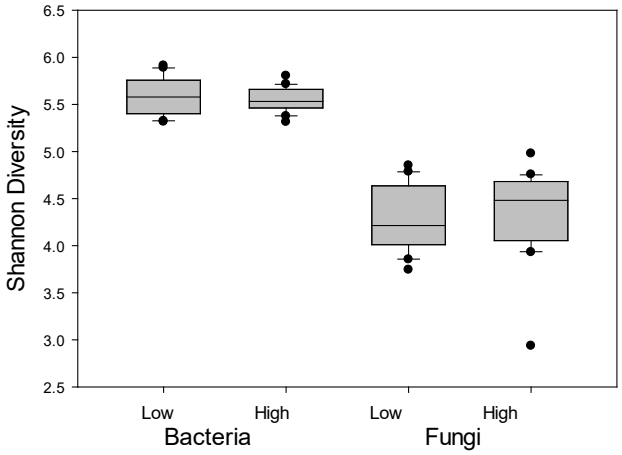

Figure 4. There was no statistically significant difference ($p < 0.05$, $n = 40$) in the diversity of bacterial and fungal communities found in soils categorized as having a low or high incidence of Verticillium wilt.

Across the 40 soils, 68 different PLFAs occurred, although most samples had about half that number with many of those PLFAs in common. Consequently, the ability to discriminate among microbial communities using PLFAs is somewhat limited. Of the variables examined, PLFAs found a significant difference in microbial community composition only by region of origin (Table 1).

Sequencing allowed for much greater resolution of bacterial and fungal communities because about **8,500 bacterial taxa and 10,000 fungal taxa** were identified. Each taxon was defined as an "amplicon sequence variant" and can roughly be considered equivalent to a species of bacteria or fungi. Analysis of the sequence data showed that both bacterial and fungal communities varied by region, soil order, and CASH texture class, with differences among regions and soil textures being most pronounced (Table 1). Bacterial communities differed significantly between low and high disease incidence soils, but fungal communities did not (Table 1).

One can visualize the differences between bacterial communities from soils with low and high disease incidence in an ordination diagram (Fig. 5). The closer two data points are in ordination space, the more similar their community composition. This nonmetric multidimensional scaling ordination captured over 83% of the variation in bacterial community composition with the two axes, thereby providing an excellent representation of the variation in the data. The bacterial communities are widely scattered across the ordination space with a lot of overlap between soils of high and low disease incidence. Nevertheless, there is a significant difference between the two groups (see large symbols with error bars) with a

Table 1. The effect of disease prevalence, geographical region, soil order, or soil texture on microbial community structure as measured by PLFA or DNA sequencing. Exact p-values are shown when the factor had a statistically significant effect on the microbial community structure. NS—not significant ($p > 0.05$). CRB—only soils within the Columbia River Basin region.

Factor	PLFA	DNA—bacteria	DNA—fungi
Disease	NS	0.0058	NS
Region	0.0123	<0.0001	<0.0001
Soil order	NS	0.0032	0.0003
CASH texture	NS	<0.0001	<0.0001
Disease (CRB)	NS	0.0026	0.0309

tendency for communities of low disease incidence soils more prevalent in the lower left of the ordination, and those of high disease incidence soils tending towards the upper right of the ordination. The high level of dispersion is partly because data are from different regions and with different soil properties. A few soil properties that are highly correlated with the composition of the bacterial communities are plotted as vectors. Soils with high silt content tend to have a low incidence of disease whereas those with a higher pH or a larger delta respiration (the decrease in respiration between days 1 and 4 during an incubation) are associated with soils with a high incidence of disease. The vectors for extractable P and active C (permanganate-oxidizable C) are not related to disease incidence but do contribute to the overall dispersion of the bacterial community composition.

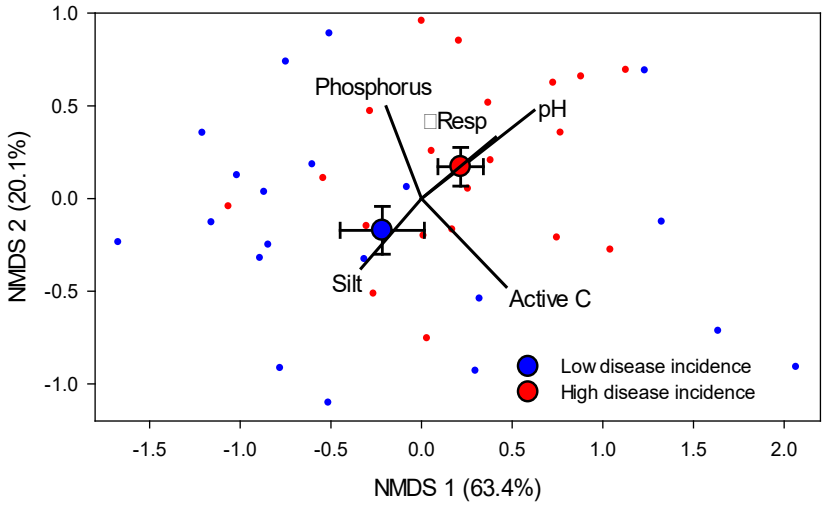

Figure 5. Nonmetric multidimensional scaling ordination of bacterial communities found in soils categorized as having a low (blue symbols) or high (red symbols) incidence of Verticillium wilt. Small symbols represent individual fields, large symbols with standard error bars represent the means of the two treatments, which are significantly different ($p = 0.0058$, $n = 40$). Vectors (black lines) represent soil properties that are significantly correlated with the structure of the bacterial communities ($p < 0.05$, $n = 40$).

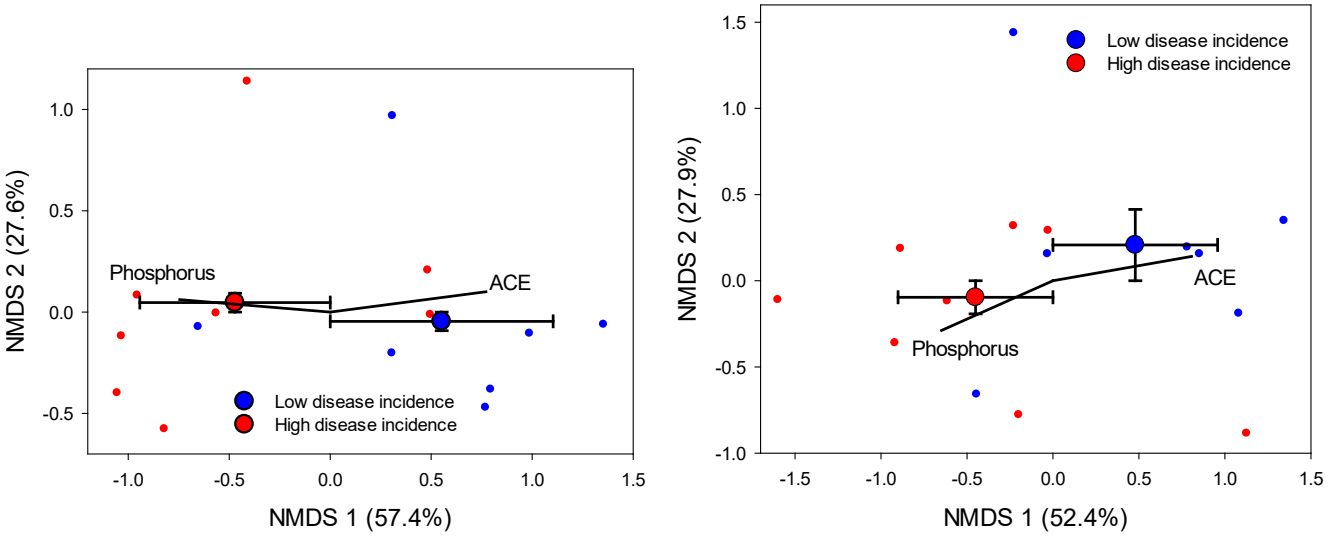

Figure 6. Nonmetric multidimensional scaling ordination of bacterial (left graph) and fungal (right graph) communities found in medium-textured soils categorized as having a low (blue symbols) or high (red symbols) incidence of Verticillium wilt. Small symbols represent individual fields, large symbols with standard error bars represent the means of the two treatments, which are significantly different ($p < 0.05$, $n = 15$). Vectors represent soil properties that are significantly correlated with the structure of the bacterial communities ($p < 0.05$, $n = 15$).

Because of the strong influence of region and soil texture on bacterial and fungal community composition, we looked more closely at the microbial communities in the medium-textured soils. Within the medium-textured soils, both bacterial and fungal communities differed between soils of low and high disease incidence (Fig. 6). In both cases, soils with high disease incidence had higher levels of extractable P, whereas those with a low incidence of disease had higher levels of ACE protein, a measure of available N.

We explored the bacterial and fungal sequencing data to see if there were significant associations of bacterial and fungal groups with disease incidence. Sequences of *Verticillium* spp. were in relatively low abundance, generally <1%, across all 40 soils with no significant difference between soils classified as low or high in disease incidence. There are at least three reasons for not finding a relationship. First, *Verticillium* persists in soil as microsclerotia, which are difficult to lyse for DNA extraction. Second, soils were placed into low and high disease incidence categories based on grower perceptions about their fields; we did not have direct measures of disease. Third, Verticillium wilt of potatoes is caused by particular pathotypes of *Verticillium* and our sequencing approach was not sensitive enough to detect pathotypes.

Because we found actinomycete biomass, based on PLFA, was higher in soils with low disease incidence, we tested whether the Actinobacteria (i.e., actinomycetes), and other bacterial phyla, differed between soils with low or high disease incidence; however, no phyla were found to be significantly different between the two types of soil. We note that sequence data represents the relative, not absolute, abundance of different taxa and that the relative abundance of actinomycete PLFAs were not significantly different between low and high disease incidence soils.

It is also possible to determine if specific bacterial or fungal taxa are indicative of low or high disease incidence soils. About 1% of bacterial and fungal taxa were found to be significantly more prevalent in either low or high disease incidence soils, but few meaningful patterns were observed. Although several Actinobacteria, Proteobacteria, and Firmicutes have been found to be antagonistic towards various pathotypes of *Verticillium*, no consistent pattern was found. For example, members of the actinobacterial Solirubrobacteriaceae family were found to be indicators of both low and high disease incidence soils. Two interesting associations did show up, however. Within the Acidobacteria phylum, species within Subgroup 6 were associated almost exclusively with high disease incidence soils whereas other Acidobacteria species were associated with low disease incidence soils. Little is known about the ecological roles of Acidobacteria, which are generally thought to be organic matter degraders. Indicator taxa that function in nitrification were associated only with high disease incidence soils.

Conclusions

This investigation had two major objectives: a) examine the association between a range of soil microbial variables and soils identified as having a low or high incidence of Verticillium wilt and b) determine how soil microbial variables are related to soil health indicators used in the Comprehensive Assessment of Soil Health (CASH).

For Objective a), certain characteristics of the microbial communities showed a statistically significant association with soils in the low and high disease incidence groups. The composition of the bacterial community, based on DNA sequencing, differentiated between the two groups of soils, despite the strong influences of region of origin and soil texture. Several bacterial taxa were associated with the low incidence soils; however, these taxa did not represent those previously known to be suppressive against soilborne pathogens and would need to be isolated to confirm the correlations observed. There was also an indication that soils with a higher microbial biomass, as measured with PLFAs, were associated with soils with low disease incidence, suggesting that practices that build up the microbial community may have a generally beneficial effect in suppressing Verticillium wilt. Finally, grouping soils by texture, as done in the CASH assessment for other soil health indicators, enhanced the ability of soil microbial characteristics to distinguish between soils with a low or high susceptibility to disease.

For Objective b), several correlations were observed between soil microbial community characteristics and CASH soil health indicators. Across soils from all three regions, many of the CASH indicators were highly correlated with bacterial community composition, including active C and extractable P, and some (pH and delta respiration) were associated with the disease state of the soils. These relationships may suggest approaches that might be used to manipulate the state of the microbial communities towards a desired endpoint.

References

Berg, G., Fritze, A., Roskot, N. and Smalla, K. (2001) Evaluation of potential biocontrol rhizobacteria from different host plants of *Verticillium dahliae* Kleb. J. Appl. Microbiol. 91:963–971.

Conn, K.L. and Lazarovits, G. (1999) Impact of animal manures on verticillium wilt, potato scab, and soil microbial populations. Can. J. Plant Pathol. 21:81–92.

Cook, R.J. and Baker, K.F. (1983). The Nature and Practice of Biological Control of Plant Pathogens. APS Press, St. Paul, MN.

Davis, J.R., Huisman,O.C., Westermann, D.T., Hafez, S.L., Everson, D.O., Sorensen, L.H. and Schneider, A.T. (1996) Effects of green manures on verticillium wilt of potato. Phytopathol. 86:444–453.

Hills, K., Collins, H., Yorgey, G., McGuire, A. and Kruger, C. (2020) Improving soil health in Pacific Northwest potato production: a review. Am. J. Potato Res. 97:1–22.

Inderbitzin, P., Ward, J., Barbella, A., Solares, N., Izyumin, D., Burman, P., Chellemi, D.O. and Subbarao, K.V. (2018) Soil microbiomes associated with Verticillium wilt-suppressive broccoli and chitin amendments are enriched with potential biocontrol agents. Phytopathol. 108:31–43.

Johnson, D.A. and Dung, J.K.S. (2010) Verticillium wilt of potato – the pathogen, disease and management. Can. J. Plant Pathol. 32:58–67.

Lazarovits, G. (2010) Managing soilborne disease of potatoes using ecologically based approaches. Am. J. Potato Res. 87:401–411.

Powelson, M.L. and Rowe, R.C. (1993) Biology and management of early dying of potatoes. Annu. Rev. Phytopathol. 31:111–126.

Uppal, A.K., El Hadrami, A., Adam, L.R., Tenuta, M. and Daayf, F. (2008) Biological control of potato *Verticillium* wilt under controlled and field conditions using selected bacterial antagonists and plant extracts. Biol. Control 44:90–100.

Wiggins, B.E. and Kinkel. L.L. (2005) Green manures and crop sequences influence potato diseases and pathogen inhibitory activity of indigenous streptomycetes. Phytopathol. 95:178–185.