

## BIOAg Project FINAL Report:

“Building soil quality, enhancing soil microbial function, and alleviating chlorosis in Concord grapes with inter-row cover crops”

### Principal Investigator:

Tarah Sullivan: Assistant Professor of Soil Microbiology, Crop and Soil Sciences, Washington State University, PO Box 646420, Pullman WA 99164. [t.sullivan@wsu.edu](mailto:t.sullivan@wsu.edu)

### Co-Investigator:

Joan Davenport: Professor of Soil Fertility, Crop and Soil Sciences, Washington State University, Irrigated Agriculture Research and Extension Center, 24106 N. Bunn Road, Prosser, WA 99350. [jdavenp@wsu.edu](mailto:jdavenp@wsu.edu)

### Abstract:

Leaf yellowing, or chlorosis, occurs on more than 50% of the Concord vineyards in central WA, resulting in significant reductions in vine size, uniformity, productivity, and eventually vine death. The yellowing of the leaves resembles classic Fe-deficiency chlorosis, however many studies have attempted without success to determine the precise cause and effective treatment of this type of chlorosis. All work, to date, supports the concept that some aspects of soil quality, specifically soil biogeochemistry, are involved in grape chlorosis in central Washington State. Through 2016-2017, we began testing a panel of four cover crops known to enhance soil quality through a variety of mechanisms, such as siderophore production, nitrogen fixation, or simply building soil organic matter. Through these functions, we hypothesized cover-cropping would enhance soil biological activity and serve as a means to manage beneficial soil microbes. We initiated the study with two different vineyards in 2016. However, the chlorosis in the second vineyard became so severe, the grower removed the entire vineyard in early 2017 and planted the area to watermelons. Therefore, we continued work in one vineyard, where we tested five replicate plots of each cover crop and a grower-control (extant vegetation) in a complete randomized block design. Measures of chlorosis severity, canopy density, leaf tissue nutrient content, soil pH, Fe content, and microbial community functions were undertaken. Initial work focused on developing a method to easily screen whole vineyards for microbial Fe-chelating abilities (siderophore activity). After this method was optimized, it was successfully applied along with a suite of microbial community functions to determine the success of cover cropping towards increasing soil health and reducing vine chlorosis. All microbial community activity levels were associated with increased soil health when measured directly under the cover crop. Additionally, vine rooting zone microbial siderophore activity was significantly associated with increased canopy density. However, it was unclear if cover cropping and these increased microbial activities led to decreased chlorosis over the course of this short-term study, particularly with only one vineyard.

## Project Description:

The global objective of this ongoing research is to determine how the structure and function of the Concord rooting zone microbial community varies between “healthy” and “chlorotic” vines and how to best manage those communities for vine health. Within the context of that objective, we intend to determine aspects of soil quality and fertility enhanced by inter-row cover cropping which produce microbial communities vital to concord Fe nutrition. Our more specific research objectives follow:

1. Evaluate different cover crop plants for their potential to increase soil quality (in terms of microbial community function, specifically related to nutrient turnover and bioavailability), and decrease chlorosis severity in associated Concord vines.
2. Determine differences in soil sampling within the vine row or beneath the cover crop, as well as during different physiological stages of the vine during the growing season.

2016: We were able to begin work with planting cover crops and obtaining the first season of data at two different vineyards, Schipleroot and Green Giant in late 2015. At each site, we had the completely randomized block design, with 4 different cover crops (rye, barley, wheat, and a wheat/vetch mix) and one grower-control (extant vegetation). In 2016, measures of soil Fe content were determined with PRS probes as well as DTPA soil extractions, and microbial siderophore activity was determined through development of a new microtiter method. Chlorosis severity was determined visually, leaves were collected for tissue nutrient analyses, and canopy density measures were taken at veraison.

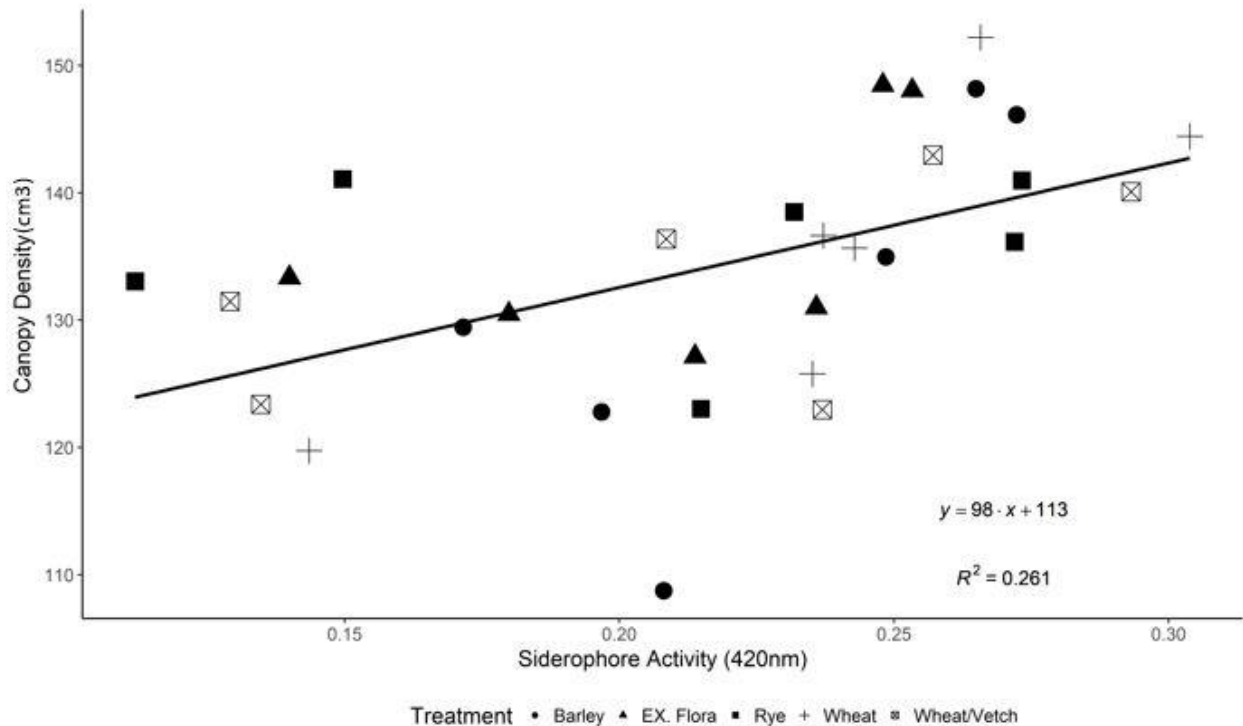
2017: The Green Giant vineyard suffered severe chlorosis losses, to the point that the grower removed the entire vineyard and converted to watermelon production for 2017 and 2018. With the loss of one vineyard, we determined to take additional measures and additional timepoints at the remaining vineyard. We returned to the plots in the Schipleroot vineyard at bloom and veraison, sampled all the same soil and vine metrics from 2017, plus three additional microbial enzyme activities (alkaline phosphatase, beta-glucosidase, and polyphenol oxidase) to aid in determinations of nutrient cycling and soil health throughout the growing season. In general, cover crop establishment for rye and barley were not as robust as the wheat and wheat/vetch mix and we found the grower-control was often a mixture of weedy species and wheat, which made clear distinctions between treatments often difficult.

## Outputs:

### Overview of Work Completed:

In 2016, we were able to develop a new method for rapid evaluation of soil microbial capacity to chelate Fe, a key nutrient in grapevine chlorosis, by siderophore activity measured by CAS color change (see JoVE article citation under “Publications”). While soil pH, soil

moisture, and other edaphic factors were not different under healthy vs chlorotic vines (the vineyard soils were very uniform), we were able to clearly correlate siderophore activity, as measured by our newly developed technique, with grapevine canopy density, regardless of cover crop treatment (Figure 1).



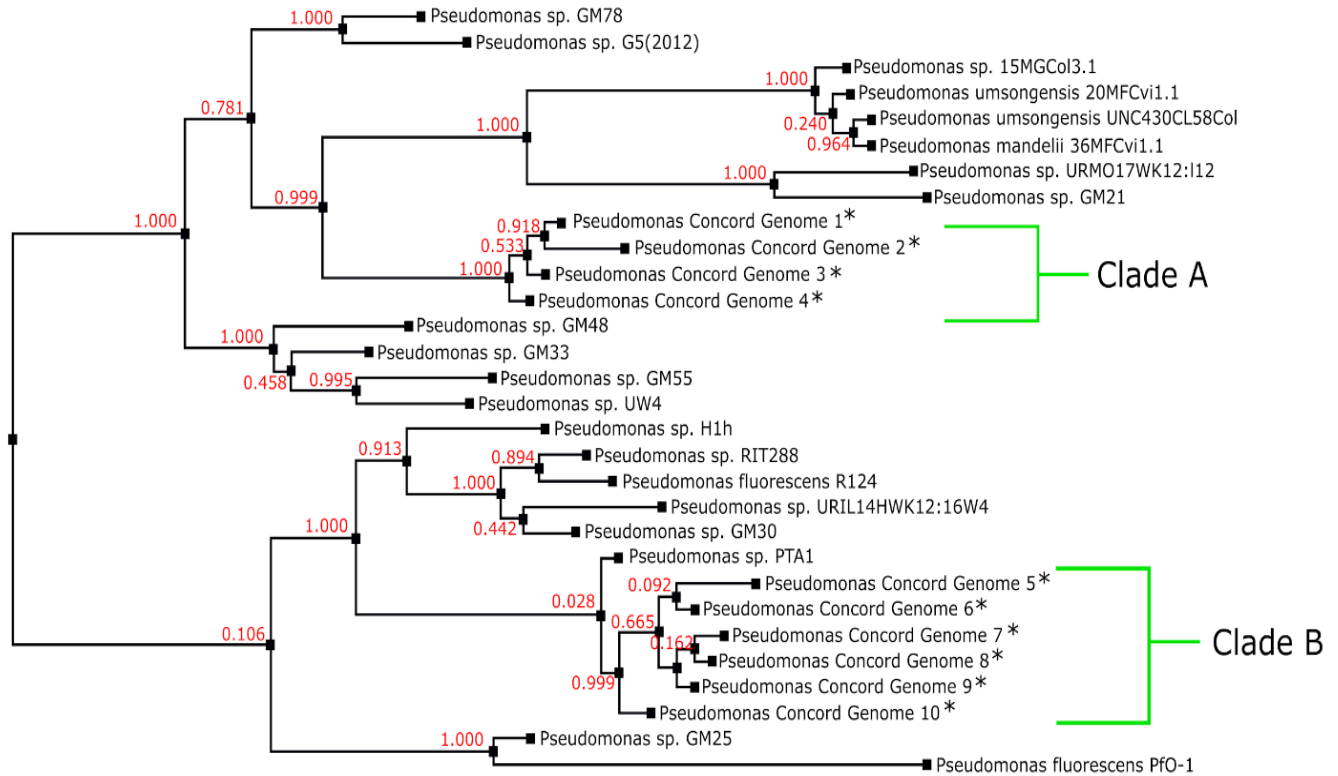
**Figure 1.** Significant correlation between ‘Concord’ grapevine canopy density and the rooting zone microbial community siderophore activity as measured by our microtiter technique.

While the R-squared value is not particularly high ( $R^2 = 0.261$ ) and indicates other factors at work in synergy with siderophore production, the correlation was highly significant ( $p < 0.001$ ) and seems to point towards an important role that siderophore production could play in overall vine health and microbial community function in the first year of the study.

We therefore decided to pursue questions as to the capacity of our novel, high-throughput CAS microtiter plate assay to be useful for identifying siderophore-producing bacteria from grapevine soils. We utilized a subset of 16 wells from various microtiter plates and sampled DNA for sequencing. From 10 of these wells, we were able to produce whole genomes that are 96-100% complete.

Sequenced whole genomes were most closely related to *Pseudomonas fluorescens*, a common plant growth promoting organism often found in the rhizosphere of many crop plants, exerting many plant-beneficial traits towards pathogen protection, increased health and vigor of the crop. The genomes recovered from the grapevine roots in the current study separated into two clades, with genomes 1-4 representing clade “A” and genomes 5-10 representing clade “B” (Figure 2). The phylogenetic distance between the two clades suggests two distinct

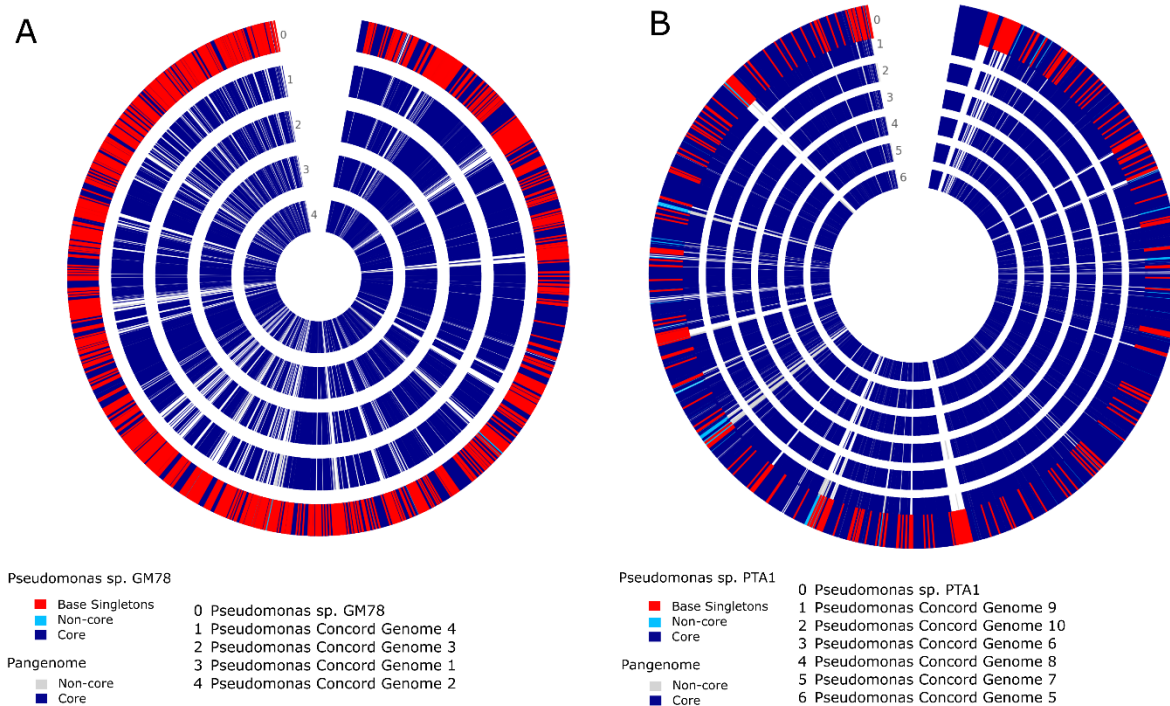
lineages. Additionally, divergence events within each clade suggest sequencing of several distinct taxa. However, there were no clear correlations between clade identity and cover crop treatment, soil measurements, or vine chlorosis rank (Figure 2).



**Figure 2.** Phylogenetic tree of the 10 newly constructed genomes with two distinct clades (A and B) located within the *Pseudomonas* genus. Generated by Insert Genome into Species Tree app in KBase using the 20 closest relatives in the public database. Numbers in red are local support values. Asterisks indicate genomes sequenced in this study.

Comparative genomics analysis of the clades revealed a total of 55 siderophore-related protein families were identified in clades A and B with seven families unique to Clade A, 15 families unique to Clade B, and 29 families shared between the clades. Pangenomic analysis of the CAS-selected cultures revealed a variety of siderophore and other protein families related to Fe nutrition and transport.

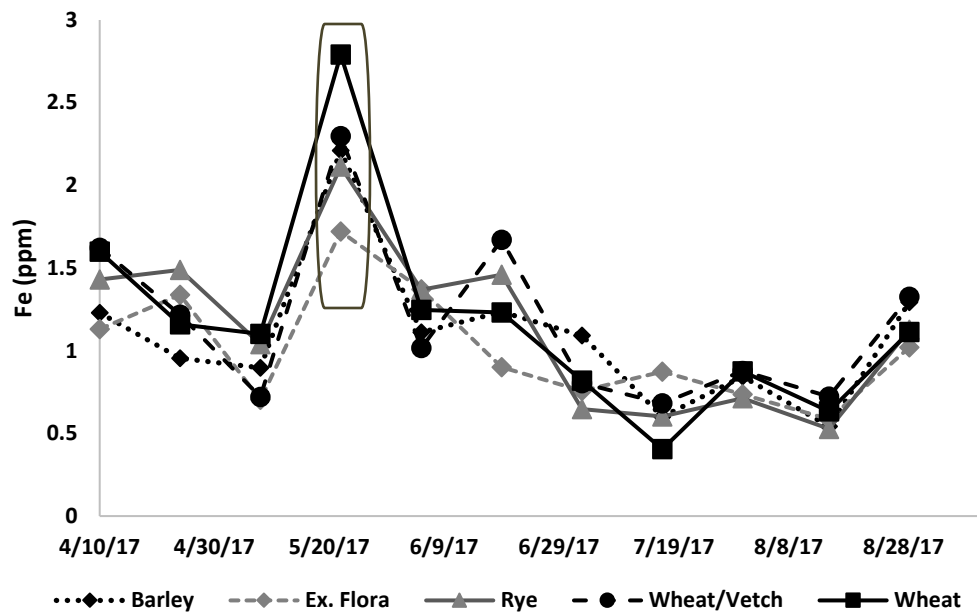
A substantial functional overlap was identified between two phylogenetically distinct clades including the capacity to express proteins related to the biosynthesis, transport, and/or perception of the three major groups of siderophores (i.e. carboxylates, hydroxamates, and catecholates), as well as mixed-type siderophores which have two or more iron binding functional groups (Aznar and Dellagi, 2015). The majority of the siderophore-related protein families found in the ten sequenced genomes belonged to the mixed-type category and included protein families related to pyoverdine and aerobactin biosynthesis, utilization, and regulation (Figure 3).



**Figure 3.** Pangenome analysis of Clades A (A) and B (B). Red indicates base singletons, sky blue represents non-core genes, with respect to the nearest identified relative organism, gray represents non-core genes within the entire pangenome, and dark blue represents core genes.

However, while these data are incredibly useful for understanding the microbial ecology and siderophore activity at the gene-level within the rhizosphere of grapevines, they tell us little as to the impacts of cover cropping on overall soil health and other microbial functions involved in nutrient turnover. We therefore expanded the study to include other microbial community activities in the second year.

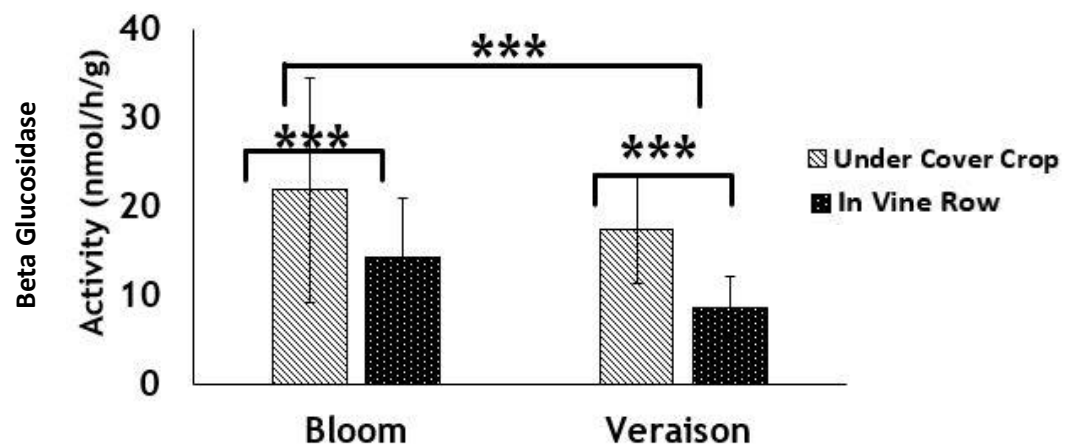
In 2017, we only had the Schipleroot vineyard, but included sampling time points during both bloom and veraison, as well as sampling locations under the cover crop itself, and locations strictly within the vine rows. Additionally, we implemented EDTA coated ‘plant root simulating’ (PRS) probes along the vine rows to monitor soluble Fe throughout the season. Unfortunately, a clear pattern of Fe availability did not emerge with regard to cover crop treatment, with a great deal of variability over the season (Figure 4). Although the decreasing trend suggests that actively growing vines deplete the concentrations of bioavailable Fe through the season, with the lowest values sometime after Veraison (Figure 4).



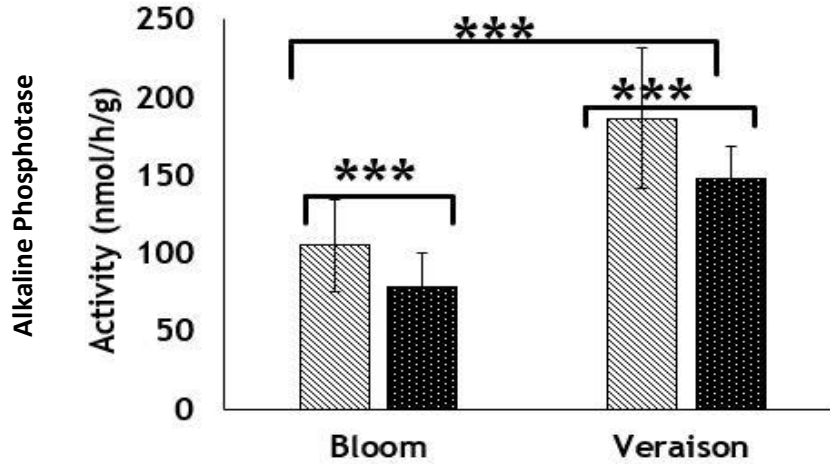
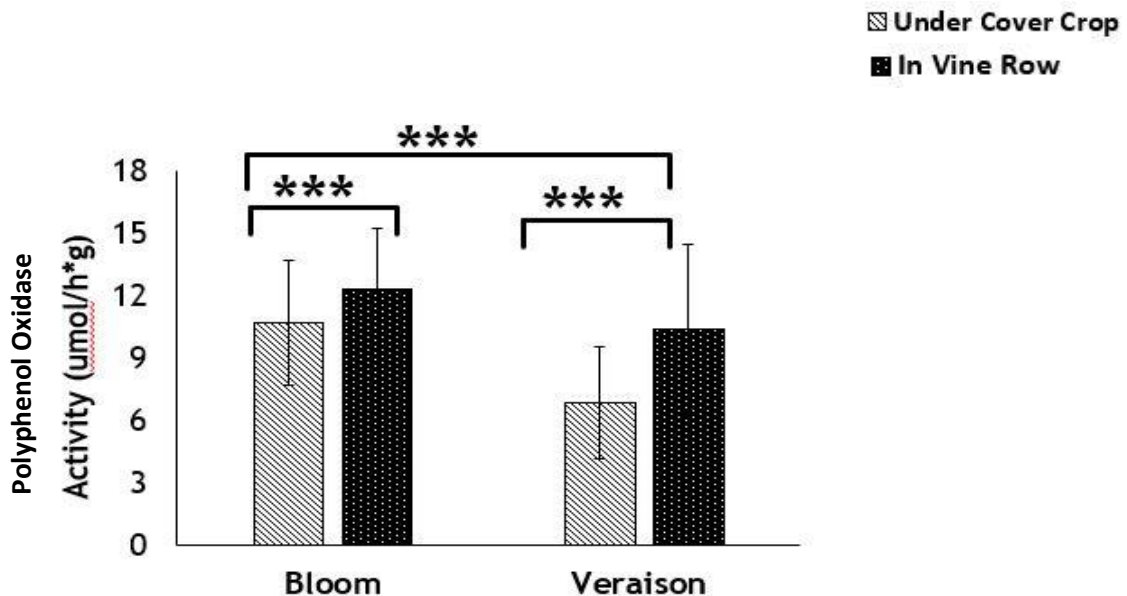
**Figure 4.** Soil Fe concentrations (ppm) extracted from EDTA coated PRS probes at the Schipleroot vineyard during the growing season of 2017.

Soil microbial enzyme activity was significantly different between sampling locations (under the cover crops vs within vine rows) and physiological stages (bloom and veraison) (Figure 5A-C). Enzyme activity was higher under the cover crops than in the soil from in within vine rows, except for polyphenol oxidase enzyme activity (Figure 5C). No significant differences of specific cover crop treatments were detected, however the higher activity levels under cover crop locations may be indicative of increased nutrient turnover and soil health directly under the cover crops themselves without transfer of this effect to the vine row.

**A.**

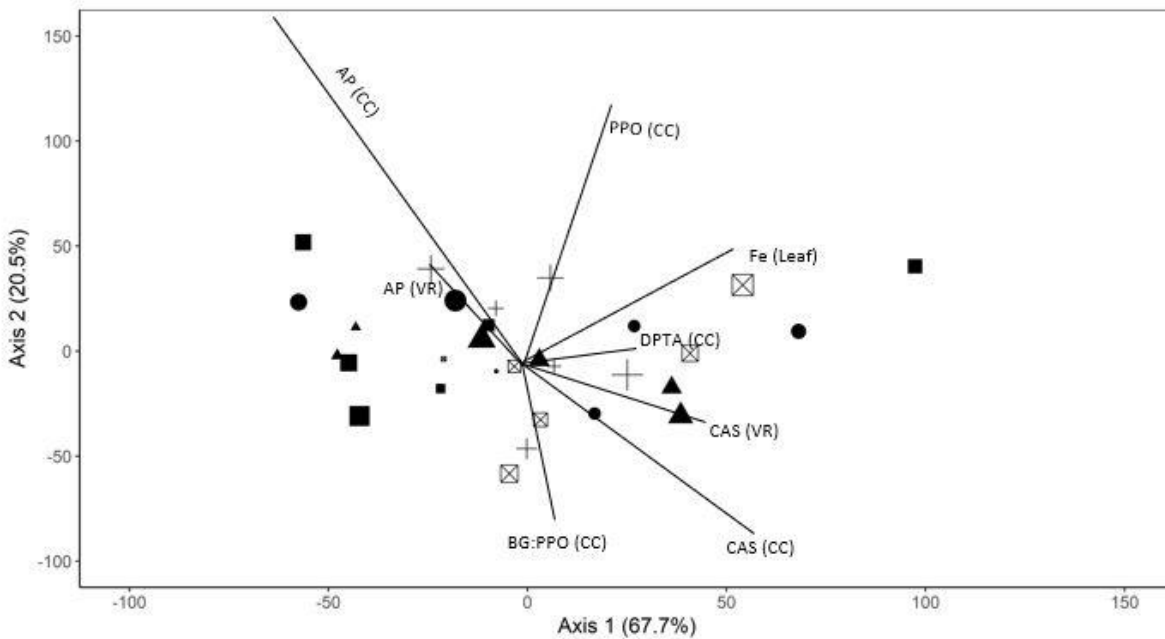
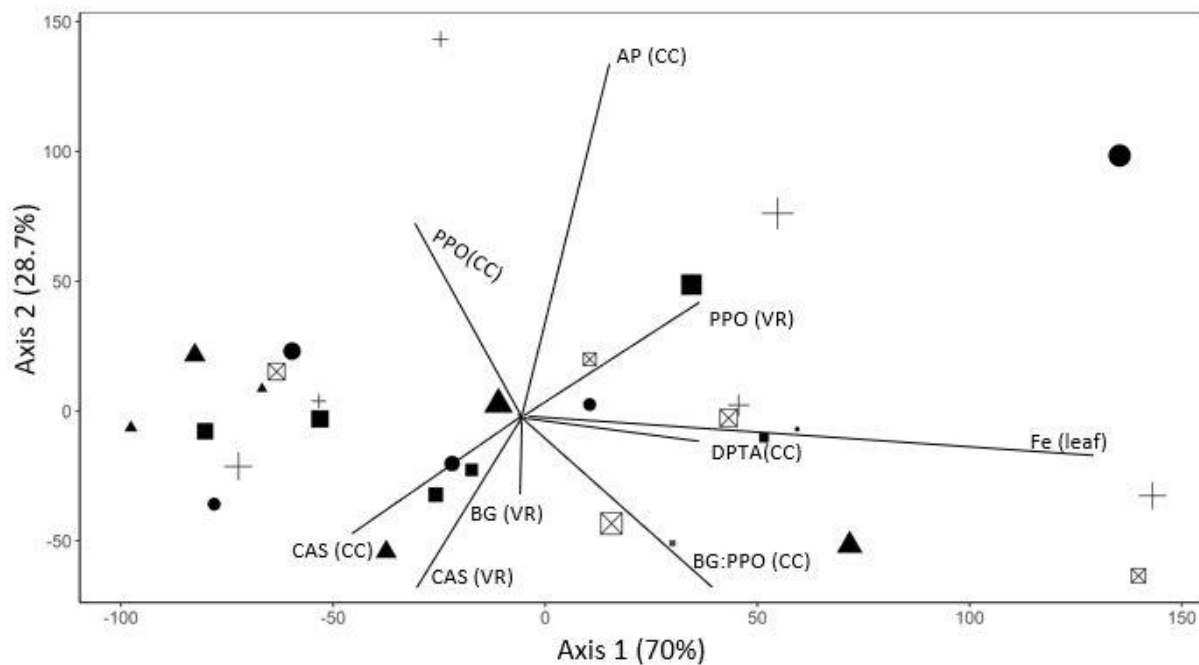




**B.****C.**

**Figure 5.** Soil microbial enzyme activities in 2017 comparing activity levels for beta-glucosidase (A), alkaline phosphatase (B), and polyphenol oxidase (C), at bloom and veraison, as well as between sampling locations (under the cover crop vs. within vine rows).

Overall, however, we had to combine all the measures of microbial activity with all the above-ground measures of plant health and vigor in order to get a better picture of how these activities may all be linked. To do that, we applied Principal Coordinates analysis to all the data we collected in 2017 at each physiological stage in vine development and determined that a huge degree of variability is explained when all the microbial activities are combined (Figure 6). The strongest determinants of chlorosis, however, were slightly different between bloom (Figure 6A) and veraison (Figure 6B). While alkaline phosphatase and CAS (siderophore) activity are both clearly very important at both growth stages, leaf tissue Fe becomes a prominent indicator of vine health at veraison, and not during bloom.

**A.****B.**

**Figure 6.** Principal Coordinates analysis of both above- and below-ground data from 2017 Schipleroot vineyard during bloom (A) and veraison (B), with treatments differentiated by symbol shape and chlorosis status indicated with symbol size (larger symbols indicate increased severity of chlorosis.) Line vectors indicate the degree to which each environmental variable helped explain the health status of the vine, with longer vectors indicating greater R-squared values.



## Discussion and Conclusions:

As mentioned above, a new technique for the metal-chelating ability of the soil microbial community was developed and applied for this project. We assessed several aspects of microbial community activities and determined that not a single measure alone could help predict the health or chlorotic state of the vine, but rather all the activity measures combined. This is consistent with our previous work in May Nursery (Lewis et al., 2018) which examined the composition of the rooting zone microbial community and found that there were many plant growth promoting bacteria present in the rhizosphere of healthy vines, and those organisms were essentially absent in the rooting zone of chlorotic vines. The beneficial activities of these organisms such as enzyme activities to promote nutrient cycling and Fe bioavailability were part of the bigger picture in our final analyses and showed that a healthy microbial population is necessary for healthy vines. When that community shifts away from the plant growth promoting community, the enzyme activities and Fe-chelating abilities of the new rhizosphere microbial community do not support healthy vine growth.

It appears that cover cropping undoubtedly increases the beneficial effects of microbial activities and spurs nutrient turnover, even increasing soil health, but that this effect may not be significantly different between cover crop types. It also may not be an effect that translates into increased vine health, as the effects appear to be restricted to directly below the cover cropped area and not to within the vine row. However, many years of cover cropping could eventually have impacts that reach the vine row and impact vineyard soil health, effects that would not have been evident in a short-term study such as ours.

## Publications:

1. BIOAg 2017 Symposium Poster, on method development.
2. ASEV 2017 National Meeting Poster, on rhizosphere phosphatase activity.
3. SSSA 2017 National Meeting Poster (Oct 2017), on first year data.
4. Washington Winegrowers Convention and Trade Show Poster (Jan 2018).
5. Washington State Grape Society Conference Presentation (Nov 2018).
6. Anjuman Islam's Masters Thesis: To be completed February 2019.
7. Peer-reviewed publications:

Lewis, R., LeTourneau, M.K., Davenport, J. & T.S. Sullivan, 2018. 'Concord' grapevine nutritional status and chlorosis rank associated with fungal and bacterial root zone microbiomes. *Plant Physiology and Biochemistry*, 129:429-436.

Lewis, R., Islam, A., Dilla-Ermita, J.C., Hulbert, S.H., and T. S. Sullivan. High-throughput siderophore screening from environmental samples: plant tissues, bulk soils, and rhizosphere soils. *Journal of Visualized Experiments* (In Press: <https://www.jove.com/in-press>).

Lewis, R., Opdahl, L., Islam, A., Davenport, J., and T. S. Sullivan. Comparative genomics, siderophore production, and iron scavenging potential of root zone soil bacteria isolated

from 'Concord' grape vineyards. *Microbial Ecology* (Accepted with minor revisions. Revised and resubmitted Nov 21, 2018).

Islam, A., Davenport, J., and T.S. Sullivan. Cover cropping improves soil health and microbial activity in chlorotic 'Concord' vineyards in the alkaline, irrigated grape vineyards in central Washington. *American Journal of Enology and Viticulture* (to be submitted Feb 2019).

## Impacts

- **Short-Term:** This project has increased our understanding of the importance of rhizosphere siderophore production by root-associated microorganisms and the genetics and genomics of the interactions of these organisms with the grapevine rooting system. We have also increased understanding of the importance of cultivating a cover crop for proper vineyard floor management can lead to increased microbial activities and nutrient turnover in the zone where the cover crop is planted. The results of this study can be leveraged for future funding from a variety of sources and lay the groundwork for similar studies in other perennial crops.
- **Intermediate-Term:** The knowledge generated from this BioAg project will assist organic and sustainable producers to develop economically feasible and long-term solutions to vineyard floor management with cover crops to enhance soil health. Additionally, the genes associated with microbial siderophore production that may be most closely associated with vine health, can be targeted across vineyards and new management strategies developed in order to further enhance the expression of those genes and lead to healthier vines through management of the rhizosphere microbial communities.
- **Long-Term:** Increasing crop nutrient uptake and overall vigor and yield by long-term sustainable management of the soil microbial community is technology that can transfer to many different annual and perennial cropping systems. This project takes us one step closer to understanding the mechanisms of metal and micronutrient enhancement in crops.

**Graduate students funded:** ANJUMAN ISLAM, MS IN SOIL SCIENCE

**Recommendations for future research:** The study would be greatly enhanced under longer-term conditions and with more vineyards across the state. Additionally, being able to diversify the types of cover crops even further and attempt to plant the cover crops as a solid mat throughout the vineyard floor instead of in strips between vine rows, would

likely be a huge benefit to the vines and vine health. Further exploration of the siderophore genetics and what controls expression may also be required.