ANNUAL REPORT

I. Project Title: **Characterization of Microbial Communities in Vineyard Soils**

II. Principal Investigator: Kate M. Scow, Department of Land, Air and Water Resources, University of California, One Shields Ave., Davis, CA, kmscow@ucdavis.edu

Collaborators:
Ken Graham (technician), Department of Land, Air and Water Resources, University of California, One Shields Ave., Davis, CA 95616

L.E. Gardner (grad student), Department of Viticulture and Enology, University of California, One Shields Ave., Davis, CA 95616

III. Summary

The purpose of this study was to characterize soil microbial communities in vineyard soils and to determine if particular kinds of microbial communities are associated with specific wine regions or soil properties. Representative soil samples were collected from twelve Pinot Noir vineyards in Anderson Valley, Russian River Valley, Los Carneros, Chalone, and Santa Maria Valley at the time of first bloom. These samples were analyzed for microbial community biomass and composition using phospholipid fatty acid (PLFA) analysis. A subset of samples were also analyzed at times of veraison and harvest. PLFA analysis provides a measure of living microbial biomass, a "fingerprint" of the soil community; and biomarkers for specific groups of microorganisms. Physical and chemical properties of the soils were also measured, including pH, electrical conductivity, cation exchange capacity, total nitrogen, total carbon, carbonate carbon, sulfate, particle size distribution, total phosphorus, potassium, nitrate nitrogen, and ammonium nitrogen. Microbial biomasses ranged from 72.2 to 234 nanomoles per gram soil and the total number of fatty acids detected ranged from 39 to 56. Although some samples from within a particular region were similar to one another, overall there was not a strong relationship between wine region and a particular kind of microbial community. At the subset of sites sampled at different times over the growing season, seasonal changes in microbial communities were detectable, but smaller than the differences between sites. There appeared to be small seasonal changes in the community that were common to all soils. The texture of the soils reflected considerable variation with clay contents ranging from 7% to 38% and sand contents from 23% to 68%. Soil pH also varied substantially from 4.8 (in pastureland adjacent to a vineyard) to 7.4. Total organic carbon ranged five-fold from 0.82% to 4.85%. Ammonium levels were for the most part lower than 13 ppm and nitrate less than 18 ppm. As we continue to catalog differences in microbial communities across a larger set of vineyard soils and at additional times, we will have better information to answer

questions such as whether there are unique traits common to all vineyard communities, or whether region has a stronger influence than crop on microbial communities. Also this information will help us begin to understand how vineyard management practices and seasonal fluctuations affect microbial community composition.

IV. Objectives and Experiments Conducted to Meet Stated Objectives

1. Determine differences in microbial community structure and microbial biomass among vineyard soils using phospholipid fatty acid (PLFA) analysis.

2. Assess the relative importance of environmental factors affecting microbial community structure in vineyard soils.

Objective 1: Representative soil samples were collected at bloom from twelve Pinot Noir vineyards in five wine-growing major regions during June 1998 and analyzed for microbial community size and composition using phospholipid fatty acids (PLFA) analysis. Sites were chosen to include soils typical of each region based upon recommendations of growers and university extension specialists. The vineyards studied are shown in Table 1.

Table 1. Vineyards Included in Study

Location	Vineyards	Abbreviation
Anderson Valley (Mendocino Co.)	Scharffenberger Estate Vineyard	AV-1
	Roederer Estate Vineyard	AV-2
	Handley Cellars	AV-3
Russian River Valley (Sonoma Co.)	Vino Farms (Zamora)	RRV-Za
	Steve Kistler Vineyard (Goldridge)	RRV-Go2
Los Carneros (Napa/Sonoma Co.)	Carneros Estate Vineyard – Cuvaison (Diablo)	CAR-Di1
	Gloria-Ferrer Circle Bar Ranch (Diablo)	CAR-Di2
	Tula Vista Ranch- Buena Vista Winery (Haire)	CAR-Ha
	Tula Vista Ranch- Dryland Sheep Pasture (Haire)	CAR-HaN
Chalone (Montery Co.)	Chalone Vineyard (McCoy)	CHA-Mc

Santa Maria Valley (Santa Barbara Co.)	Byron Vineyards (Pleasanton)	SMV-Pl
	Cambria Vineyards (Elder)	SMV-El

Figure 1 shows a map of western United States and designates the locations of the vineyards included in this study. Also, shown is the Willamette Valley in Oregon from which Pinot Noir soil samples were also collected for another study.

PLFAs were extracted from whole soil samples, fractionated, methylated, and analyzed by gas chromatography. The data were analyzed using multivariate statistics to determine relationships between the samples and to identify which fatty acids contributed to the observed relationships. An analysis of variance (ANOVA) was performed on selected fatty acids.

Objective 2: Representative soil samples collected from all twelve vineyards were analyzed for the following by the University of California Division of Agriculture and Natural Resources (DANR) Analytical Laboratory according to their Methods of Soil Analysis: pH, EC, cation exchange capacity, total nitrogen, total carbon, carbonate carbon, sulfate, particle size distribution, total phosphorus, potassium, nitrate nitrogen, and ammonium nitrogen.

V. Summary of Major Research Accomplishments and Results

Objective 1:
There are numerous methods with which it is possible to characterize microbial communities in soil. All the methods can be grouped into 3 major categories on the basis of the type of information they generate: i) counts; ii) activity; and iii) information on cellular constituents. Counting methods include plate counts and direct microscopy, both of which are commercially available services. A weakness of plate counts is that the majority of bacteria and particularly fungi cannot be grown on plates. Direct counts provide very limited data about community composition and the numbers are confounded by the presence of labile organic material. Activity measurements include quantifying the presence or rate of virtually any process that microorganisms carry out (e.g., respiration, nitrification, etc.). Weaknesses of activity methods are that microbial contributions sometimes cannot be separated from those of plants and animals; only net process rates can be measured whereas some of the products being generated will be consumed immediately; and activity measures provide little information about the types of organisms involved. Measurements of cellular constituents include simple measures of C in microbial biomass, as well as more complex determinations of lipids (e.g., phospholipid fatty acids or PLFA) and nucleic acids (e.g., DNA, RNA). Numerous variations of these techniques have been developed in the past decade for use in characterizing entire microbial communities, many of which bypass isolation of individual microbial strains. These approaches also have their weaknesses but are constantly under

further development. In this project, we used PLFAs to characterize vineyard soil communities.

PLFAs are integral components of cell membranes and rapidly metabolized when a cell dies in soil; therefore, they provide a measurement of living organisms. Principle types of PLFAs are defined on the basis of chain length, degree of unsaturation, and presence of substituents (e.g., methyls, hydroxyls, cylopropane rings. There are three ways in which PLFA data can be used to provide information about microbial communities: i) total PLFA provides a measure of viable microbial biomass, ii) the entire PLFA profile can be used as a "fingerprint" of the soil community; and iii) signature lipids can be used to detect specific subgroups within the community: e.g., sulphate reducers, methane oxidizing bacteria, mycorrhizal fungi, and actinomycetes.

The total amount of PLFA, an indicator of total microbial biomass extracted from these samples (plus a minor amount of plant biomass), ranged from 72.2 to 234 nanomoles of fatty acids per gram dry weight of soil (Table 2). The highest biomass was measured in the Scharffenberger vineyard sample and in the Tula Vista pasture sample. The lowest biomass was measured in the Carneros, Steven Kistler, and Cambria samples. The number of fatty acids detected ranged from 39 to 56. The differences between samples were highly significant ($P<0.0001$). There was no trend associated with either location or sampling date for the total amount or number of fatty acids extracted. There was a weak positive correlation ($r^2=0.58$) between microbial biomass and soil organic carbon content.

PLFA fingerprints, each of which are made up of fatty acids contributed by all dominant members of a soil's microbial community, were compared among the different soils. To compare fingerprints requires use of a multivariate statistical technique, called Correspondence Analysis (CA), which was performed on a subset of the total number of fatty acids detected in all soil samples. CA is a data analysis technique that transforms a data set containing many variables (in this case fatty acids) into a smaller set of new variables, or dimensions. Each dimension is a unique combination of all the fatty acids that explains a percentage of the total variation in the original data set. A multi-dimensional plot of these dimensions can show relationships among soil samples, reflecting both differences and similarities among different samples. CA can also identify which particular fatty acids are most important in determining the relationships among the soil samples. CA was performed on all of the samples collected on different dates and then again only on the samples collected in June.

A plot of the first two dimensions (43.5 and 19% of the variance, respectively) from the CA of all samples collected in June, including those from the Willamette Valley, Oregon, is shown in Figure 2. The Carneros samples were not closely clustered but did, for the most part, fall out on the right hand side of the x-axis. The most southern samples, from Chalone and Santa Maria, were located on the left side of the x-axis. The Willamette Valley samples were intermingled with those from California

indicating no strong, large-scale regional trend. Overall there appeared to be no clear relationship between wine region and microbial community composition.

The CA of those samples that were collected at bloom, veraison and harvest (including Carneros and Willamette Valley samples) is shown in Figure 3. The first two components explained 73% of the variation in the data; however the trends associated with time did not appear to fall along either of the first two axes. In general, samples from each location tended to group together regardless of the date collected, which suggested that differences over time were smaller than differences among sites. Samples collected at harvest usually were located in the upper right side of each cluster of samples which may reflect temporal changes in the community common to all soils.

Another way of looking at the PLFA data is to compare the mass of specific lipid biomarkers among the sites. Table 2 shows the total PLFA extracted, the proportion of 10 methyl branched fatty acids, and the percentage of linoleic acid (18:2) for the samples collected in June. Fatty acids with methyl branching on the tenth carbon atom (10 methyl branched) are predominantly found in gram positive bacteria, largely actinomycetes. The fatty acid linoleic acid is a biomarker for fungi. Numbers followed by the same letter within each column are not significantly different at $P<0.001$.

Table 2

SAMPLE	Total PLFA (nmoles/g soil)	10-methyl branched (% of total)	18:2 (% of total)
Scharffenberger	234.4 a	0.055 c d e f	3.74 a b c d
Roederer	141.9 b c d	0.061 b c d	4.21 a b c
Handley	120.7 c d e	0.061 b c d	3.28 b c d
Carneros	80.8 e	0.079 a	2.5 c d
Gloria-Ferrer	164.7 b	0.064 b	4.63 a b c
Tula Vista	153 b c	0.054 d e f	3.26 b c d
Tula Vista (pasture)	215.2 a	0.060 b c d	2.11 d
Chalone	104.1 d e	0.048 f g	5.82 a
Steven Kistler	82.6 e	0.062 b c	5.01 a b
Vino	114.3 c d e	0.056 c d e	3.03 b c d
Cambria	85.1 e	0.048 e f g	4.82 a b
Byron	171.4 b	0.044 g	5.78 a

The overall difference among samples for all of the data in Table 2 were significantly different ($P<0.001$). Pairwise comparisons of total PLFA, percentage of 18:2, and 10 methyl branched, using Tukey's test, showed that there was no trend based on location or sampling date.

The particular fatty acids that contributed most to the differentiation of the vineyard samples in the correspondence analysis were identified. The Carneros samples (Di1 and Di2) had a high relative abundance of bacterial markers and a low relative

abundance of the fungal marker. On the other hand, samples from the Chalone vineyard and the Byron Vineyard in the Santa Maria Valley had a high relative abundance of fungal, but lower relative abundance of bacterial markers. The sample from the dryland sheep pasture at Tula Vista Ranch, though it had a very high total biomass, had the lowest percentage of fungal marker among all the samples. This limited piece of data, along with information we have from row crops and other grassland samples, suggest that vineyards support higher fungal populations than do some of the other cropping systems.

Objective 2:
Soils from all sites (2 replicates each) were analyzed for chemical and physical properties at the time of first bloom. The Los Carneros soils were also analyzed again at the times of veraison and harvest (designated –V and –H, respectively). Tables 3 and 4 summarize the data.

There was considerably variation in physical and chemical properties among the soils. The texture of the soils reflected considerable variation with clay contents ranging from a low of 7% (Byron) to a high of 38% (Carneros Estate) and sand contents from a low of 23% (again, Carneros Estate) to a high of 68% (Steve Kistler). Cation exchange was inversely related to sand content with the Steve Kistler Vineyard having the lowest (15.3 meq/100g) and Carneros Estate with the highest (40.2 meq/100g) values. Soil pH also varied substantially from a value of 4.8 (in pastureland adjacent to a vineyard) to 7.4. Total organic carbon ranged from 0.82% to 4.85% and the C to N ratio was approximately 10. Most soils had extractable phosphorus concentrations in the low ppm level with the exception of four soils with concentrations >25 ppm. Ammonium levels were generally lower than 13 ppm with the exception of one soil and nitrate less than 18 ppm, also with the exception of one (different) soil. Sulfate levels ranged from 5.5 to 25 ppm.

VI. Outside Presentations of Research

Presentations

Scow, K.M. 1999. A bug's story. Invited talk in Dept. of LAWR. (2/10)

Scow, K.M. 1999. Developing Relationships between Soil Communities and Soil Quality in Agroecosystems. Kearney Symposium (3/23)

Scow, K.M. 1999. Microbial blood and claws. Science Fair, Caesar Chavez Elementary School, Davis, CA. (4/28)

Scow, K.M. 1999. Microbiology of vineyard soils. Sonoma Valley Growers Association, Geyserville, CA. (5/7)

Scow, K.M. 1999. Role of microorganisms in the soil/atmospheric interface. Air Quality Workshop, Davis, CA (7/27).

Publications

Scow, K.M., and M.R. Werner. 1999. The soil ecology of cover cropped vineyards, p. 69-79. In: Ingels, C. (ed.) Cover cropping in vineyards. University of California Division of Agriculture and Natural Resources. Publication 3338. DANR, Oakland, CA.

VII. Research Success Statements

This research provides baseline information about the size and composition of microbial communities in vineyard soils. The PLFA method used in this study provides far more detailed information about community composition than do previously available microbiological methods. Such data are the first of this kind to be collected for vineyard soils. As we continue to catalog differences in microbial communities across a larger set of vineyard soils, we will have better information to answer questions such as whether there are unique traits common to all vineyard communities, or whether region has a stronger influence than crop on microbial communities. Also this information will help us begin to understand how vineyard management practices and seasonal fluctuations affect microbial community composition. In turn, we hope this information will eventually be useful in determining how knowledge of soil microbiology can benefit the development of successful and environmentally friendly vineyard management practices.

VIII. Funds Status

All funds have been expended. A renewal of this grant was awarded for the years 2000-2001.