

AVF PROGRESS REPORT-2001

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

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III. Summary

The purpose of this study was to continue the characterization of soil microbial communities in Pinot Noir vineyard soils by comparing the variability in microbial community composition over time in a specific vineyard relative to the variability in composition across many vineyards. The study also aimed to show the effect that vineyard management has on the diversity of soil microbial communities, by comparing a vineyard microbial community to an adjacent microbial community under a different land use on the same soil type.

A set of soil samples was collected from nine Pinot Noir vineyards in Anderson Valley, Russian River Valley and Los Carneros in June of 2000. These samples were analyzed for microbial community biomass and composition using phospholipid fatty acid (PLFA) analysis. In June of 1998, the microbial biomass in the soils ranged from 14 to 107 nanomoles per gram soil, as compared with a range of 11 to 85 in June of 2000. There was a substantial drop in microbial biomass in all vineyard soils between 1998 and 2000. The total number of fatty acids detected ranged from 38 to 58 for both years. Despite differences in biomass, the differences in community composition between the two years were small and usually less than differences between vineyards. Although some samples from within a particular region were similar to one another, overall there was still not a strong relationship between wine region and a particular kind of microbial community. A set of paired vineyard and adjacent land use sites are currently being identified with the help of growers.

IV. Objectives and Experiments Conducted to Meet Stated Objectives

1. What is effect of vineyard management on the diversity of soil microbial communities.

2. What is the variability in microbial community composition over time in a specific vineyard relative to the variability in composition across many vineyards.

The third objective, to compare communities under different rootstocks, was not addressed in this funding period due to insufficient funds and not yet having identified a suitable site. We are working with growers in the North Coast Viticulture working group to identify an appropriate site.

Representative soil samples were collected from nine of the Pinot Noir vineyards in three of the major wine-growing regions sampled in our previous study in 1998. In June of 2000, three replicate samples (increasing the replication in 1998) were pooled from 10--15 soil cores down to 15 cm deep. These samples were analyzed for microbial community size and composition using phospholipid fatty acids (PLFA) analysis. The vineyards studied are shown in Table 1.

Table 1. Vineyards Included in both 1st and 2nd Year Study

Location	Vineyards
Anderson Valley (Mendocino Co.)	Scharffenberger Estate Vineyard
	Roederer Estate Vineyard
	Handley Cellars
Russian River Valley (Sonoma Co.)	Vino Farms
	Swan Vineyard
	Steve Kistler Vineyard
Los Carneros (Napa/Sonoma Co.)	Carneros Estate Vineyard (Cuvaision)
	Gloria-Ferrer Circle Bar Ranch
	Tula Vista Ranch- Buena Vista Winery
	Tula Vista Ranch- Dryland Sheep Pasture

PLFAs were extracted from the 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.

V. Summary of Major Research Accomplishments and Results

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, cyclopropane 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, fungi, and actinomycetes.

The total amount of PLFA, an indicator of total microbial biomass extracted from the vineyard samples (plus a minor amount of plant biomass), ranged from 10 to 116 nanomoles of fatty acids per gram dry weight of soil over the course of two years (Table 2). (Note: the numbers reported in the 1998 Annual Report were two times those reported for the same data in our Year 2000 report. This was due to a calculation error but does not alter the conclusions of our previous report). There was a substantial drop in the biomass in all vineyards in 2000 compared to 1998. One reason for this drop may be due to large differences in precipitation patterns during the 2 years. The year 1998 was an El Nino year and had record amounts of rainfall. In contrast, the year 2000 had a substantially drier winter and spring. For both years, the Scharffenberger vineyard sample and the Buena Vista sheep pasture sample were consistently highest in microbial biomass. The number of fatty acids detected ranged from 38 to 58 in both years.

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. Increasing the replication in 2000 showed that there was a large amount of variability in microbial community composition within a vineyard. The CA plot of the data indicated that, for most vineyards given this within field variability, community fingerprints varied little between 1998 and 2000. Differences between the two years were small compared to differences between

vineyards, in many cases. The largest shifts over time were observed in the samples from Vino Farms and Handley Cellars.

When the CA Pinot Noir vineyard samples are compared to a set of vineyard soil and rhizosphere samples collected from an Oregon Pinot Noir Vineyard (also shown in 1998 Annual report), it becomes evident that the differences across years are very small relative to the differences between the CA Pinot Noir soils and the Oregon samples.

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 of 1998 and June of 2000. 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.

Table 2

SAMPLE	Total PLFA (nmoles/g soil)		10-methyl branched (% of total)		18:2 (% of total)	
	1998	2000	1998	2000	1998	2000
Scharffen-berger	116.59	27.94	0.054	0.076	0.038	0.028
Roederer	70.57	19.07	0.060	0.083	0.039	0.026
Handley Cellars	60.03	25.12	0.060	0.067	0.031	0.037
Vino Farms	56.87	20.40	0.055	0.083	0.028	0.019
Swan	14.13	10.76	0.087	0.071	0	0.027
Steve Kistler	41.08	18.97	0.061	0.056	0.046	0.088
Carneros (Cuvaion)	40.19	21.58	0.078	0.09	0.023	0.038
Gloria-Ferrer	81.93	25.64	0.063	0.06	0.042	0.040
Buena Vista (Vineyard)	76.09	29.67	0.053	0.058	0.030	0.033

Buena Vista (Pasture)	107.02	85.16	0.060	0.057	0.019	0.052
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The particular fatty acids that contributed most to the differentiation of the vineyard samples in the correspondence analysis were identified. Both years show that the Cuvaision samples had a high relative abundance of bacterial markers. The idea that Cuvaision also had a low relative abundance of the fungal marker is not supported. The sample from the dryland sheep pasture at Tula Vista Ranch, had the lowest % fungal marker in 1998, even though it had a very high total biomass. The 2000 data does not support this as a trend. We are still in the process of analyzing this set of data.

VI. Outside Presentations of Research

Presentations:

- Scow, K.M. 2000. Microbial Communities of Agricultural Soils. Invited talk at Soil, Food and People Meeting. Davis, CA. 3/28/00.
- Scow, K.M. 2000. Microbial Communities of Vineyard Soils. Invited talk at Napa Valley Vineyard Growers Meeting. Napa, CA. 6/7/00.

Publications

- Scow, K.M., E. Schwartz, M. Johnson, and J.L. Macalady. 2000. Measurement of microbial diversity. In: Encyclopedia of Biodiversity. (in press).
- Scow, K.M. 1999. Soil microbiology. In: Encyclopedia of Microbiology. Academic Press (in press).
- Fitzgerald, G., K.M. Scow, and J. Hill. 2000. Fallow season straw and water management effects on methane emissions in California rice. *Global Biogeochemical Cycles* 14:767-776.
- Calderon, F.J., L.E. Jackson, K.M. Scow, and D.E. Rolston. 2000. Microbial responses to simulated tillage in cultivated and uncultivated soils. *Soil Biol. Biochem.* 32:1547-1559.
- Song, X.-H., P.K. Hopke, M.A. Bruns, K. Graham, and K. Scow. 1999. Pattern recognition of soil samples based on microbial fatty acid contents. *Environ. Sci. Technol.* 33: 3524-3530.
- Bruns, M.A., and K.M. Scow. 1999. DNA fingerprinting as a means to identify sources of soil-derived dust: problems and potential, p. 193-205. In: Scow et al. (eds) *Integrated assessment of ecosystem health*. Lewis Publishers, Boca Raton, FL.

Sudarshana, P., J.R. Hanson, and K.M. Scow. 1999. Application of random amplified polymorphic DNA (RAPD) method for characterization of soil microbial communities, p. 223-231. In: Scow et al. (eds) Integrated assessment of ecosystem health. Lewis Publishers, Boca Raton, FL.

VII. Research Success Statements

This continued research provides additional 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 have continued to catalog differences in microbial communities across a greater period of time we have begun to answer the questions of 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

Funds have been expended.