

Microbial Communities in Vineyard Soils: Effect of Cover Crop

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Objective 3: To evaluate the effects of cover crops on soil microbial ecology

Background and Approach

Cover crops have been shown to increase microbial biomass and alter community composition in a variety of agronomic systems. These changes in microbial communities, in turn, can affect soil structure and nutrient cycling. One approach for evaluating changes in microbial communities is phospholipid fatty acid analysis (PLFA) of whole communities extracted directly from soils. 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.

This field study to evaluate effects of cover crops on vineyard microbial communities was conducted at Deer Creek Vineyard, located near the town of Sheldon in Sacramento County, in a block of Merlot grapes on 5BB rootstock. The vineyard is drip irrigated. The soil type is San Joaquin silt loam. Soil samples were collected over the course of two years, on four different dates: April 1999, July 1999, April 2000 and August 2000. Samples have been collected from soils with 4 different cover crops, plus a control plot. The five treatments were replicated four times in the field (however, for the April 2000 data set only one of the four field replicates was sampled).

Soil samples were taken from the cover crop areas and from the berms with a 2cm corer to a depth of 15cm. Data are reported as the mean of three lab replicates (except where the third lab rep was thrown out due to high variation). These samples were analyzed for microbial community size and composition using phospholipid fatty acid (PLFA) analysis. 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.

Results

1. Total biomass

The total amount of PLFA (reported in nm/g dry soil) is an indicator of total microbial biomass present in the samples (plus a minor amount of plant biomass). The average total PLFA was significantly higher in the cover crop samples than in the berm samples for all treatments (Table 1). Total PLFA was highest in the Annual clover and Native Grass treatments, the average value being 19.9 in the berm and 26.0 in the cover crop region for annual clover. The native grass samples averaged 21.0 in the berm and 28.0 in the cover crop region. Total PLFA in the barley oat and bell bean vetch treatments was lower than in the other treatments. The average values for barley oat were 16.3 in the berm and 20.2 in the cover crop samples. For bell bean vetch average values were 16.6 in the berm and 23.2 in the cover crop samples. Average total PLFA in the control plot (no cover crop planted), was the same in the berm samples (18.2) as it was in the cover crop samples (18.0). This indicates that the increased microbial biomass in the cover cropped samples is a result of the planted cover crop rather than being an artifact of location.

2. Microbial community composition

PLFA fingerprints, each of which is made up of fatty acids contributed by the 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. Some of these fatty acids are biomarkers for specific groups of organisms; however, many fatty acids contributing to the fingerprints are common to large groups and cannot provide diagnostic information about which organisms increase or decrease with cover crop treatments (specific information on some of the more useful biomarker lipids is provided below).

The CA plot of all four sampling dates (Figure 1) indicates that the microbial community fingerprints varied over time. These differences were likely associated with changes in temperature, soil moisture and duration of the cover crop treatments. The microbial communities at different times of the year (Spring and Summer) were not very well separated (because of a large amount of variability within each sample time) and showed a certain degree of overlap. This suggested that changes were primarily in the relative abundance of the same group of organisms. Over the two year period, samples shift from the left to the right of the plot, with the shift of the August 00 samples being greatest. The variation caused by sampling date is more significant than the variation caused either by the cover crop treatment or sampling

location (berm or cover crop region). The individual lipids responsible for this trend are shown in Figure 2.

CAs of each different sampling date (Figures 3-10) show that differences in microbial communities between the berm and cover crop samples is greater than differences associated with different cover crop treatments. For all data sets, the berm and cover crop region samples separate along either the first or second axis. The differentiation is likely associated with differences in rhizosphere of cover crops versus grapevines, as well due to the fact that berm samples may have had better access to irrigation water than did the cover crop samples. Differences in rhizospheres could lead to differences in soil moisture, carbon and other nutrients, competition for nutrients with plants, as well as other factors.

Among the different cover crops, the greatest differences were associated with the annual clover and native grass samples. Microbial communities associated with these two cover crops were remarkably similar to each other and grouped independently from the other cover crop samples (Figures 5&9). (CHUCK: DID THESE DIFFER IN THEIR TILLAGE PATTERNS?)

3. Specific groups of microorganisms

Another way of looking at the PLFA data is to compare the mass of specific lipid biomarkers that are indicators of particular groups of microorganisms. Table 1 & 2 show the relative proportions of biomarkers for some major groups of organisms in soil, as well as the average number of lipids and the total PLFA extracted. These biomarkers include the proportion of branched (Gram positive bacteria marker), cyclopropyl (aerobic bacteria markers), linoleic acid (18:2: fungal biomarker), and methyl branched (gram positive bacteria marker) for subgroups of the samples. Values shown are averages of samples from berm and cover crop regions of the five different cover crop treatments (Table 1) as well as averages of the berm and cover crop region samples from the four different sample dates (Table 2). (*Note: biomarker data need to be interpreted with caution; not all biomarkers are exclusive to a particular group and it is possible that some members of a particular group may not have that biomarker*).

For all cover crop treatments, the proportion of aerobic bacterial markers (cyclopropyl lipids) is higher by 6-10 % in the cover crop samples than in the corresponding berm samples (Table 1). Conversely, the fungal biomarker component (18:2w6) is always higher in the berm samples than in the corresponding cover crop samples. This trend is not caused by the presence of a cover crop (which possibly could contain this lipid), however, as the control also has a much higher percentage of fungal biomarker in the berm than cover crop samples (Table 1). There is no apparent difference in proportion of branched lipids or number of lipids between berm and cover crop region samples.

The proportion of fungal biomarker (18:2w6) is consistently higher in the spring data sets than in summer data sets. The markers for gram positive and aerobic bacteria show no apparent seasonal trends (Table 2).

Figures 2, 4, 6, 8 & 10 show those PLFAs that contributed substantially to the differentiation of the soil samples. For instance, the August 2000 samples had a high relative abundance of the

lipids designated on the right hand side of the graph (Figure 2), whereas the April 1999 samples had a low relative abundance of these lipids. Not all of these lipids are associated with specific groups of microorganisms; many are shared by a variety of groups. The reader is referred to Bossio and Scow (1998); Vestal and White (1989), and White et al. (1996) listed below for additional information.

Conclusions

- The presence of cover crop causes increases in microbial community biomass (total PLFA) in the cover crop samples relative to the berm samples. Microbial biomass does not increase in control samples for the cover crops.
- The native grass and annual clover cover crop samples have the highest relative biomass. These values are 3-4% higher than other treatments in berm & 3-10% higher than in the other cover crop samples.
- Combining all 4 sample dates together, microbial communities slightly shift towards the right from spring to summer on a CA plot. Also, the variation caused by sampling date is greater than the variation caused either by the cover crop treatment or by sampling location (berm or cover crop region).
- Within individual sample dates, the differences in microbial communities between the berm and cover crop samples is greater than differences associated with the different cover crop treatments.
- In both of the summer sample sets, microbial communities in the annual clover and native grass cover crop samples group independently from those in the other cover crop samples.
- There is a higher proportion of bacteria (as indicated by the cyclopropyl biomarker) in the cover crop than berm samples. The other bacterial biomarker (branched) did not show significant differences between berm and cover crop samples.
- There is a higher proportion of fungi (18:2w6) in the berm than cover crop samples.
- There is no difference in the number of lipids between berm and cover crop region samples, suggesting no major differences in the diversity of these communities.
- The proportion of fungal biomarker is higher in spring than in summer. Other biomarkers show no obvious seasonal trends.

References

Bossio DA, Scow KM (1998) Impacts of Carbon and Flooding on soil Microbial Communities: Phospholipid Fatty Acid Profiles and Substrate Utilization Patterns. *Microbial Ecology* 35:265-278

Vestal JR, White DC (1989) Lipid analysis in microbial ecology: Quantitative approaches to the study of microbial communities. *BioScience* 39:535-541

White DC, Pinkart HC, Ringelberg DB (1996) Biomass measurements: Biochemical approaches. In: Hurst CJ, Knudsen GR (eds), *Manual of Environmental Microbiology*. ASM Press, Washington DC, p 91-101

Figure 1: Seasonal changes in vineyard soil microbial communities
Cutoff 50 deercreek

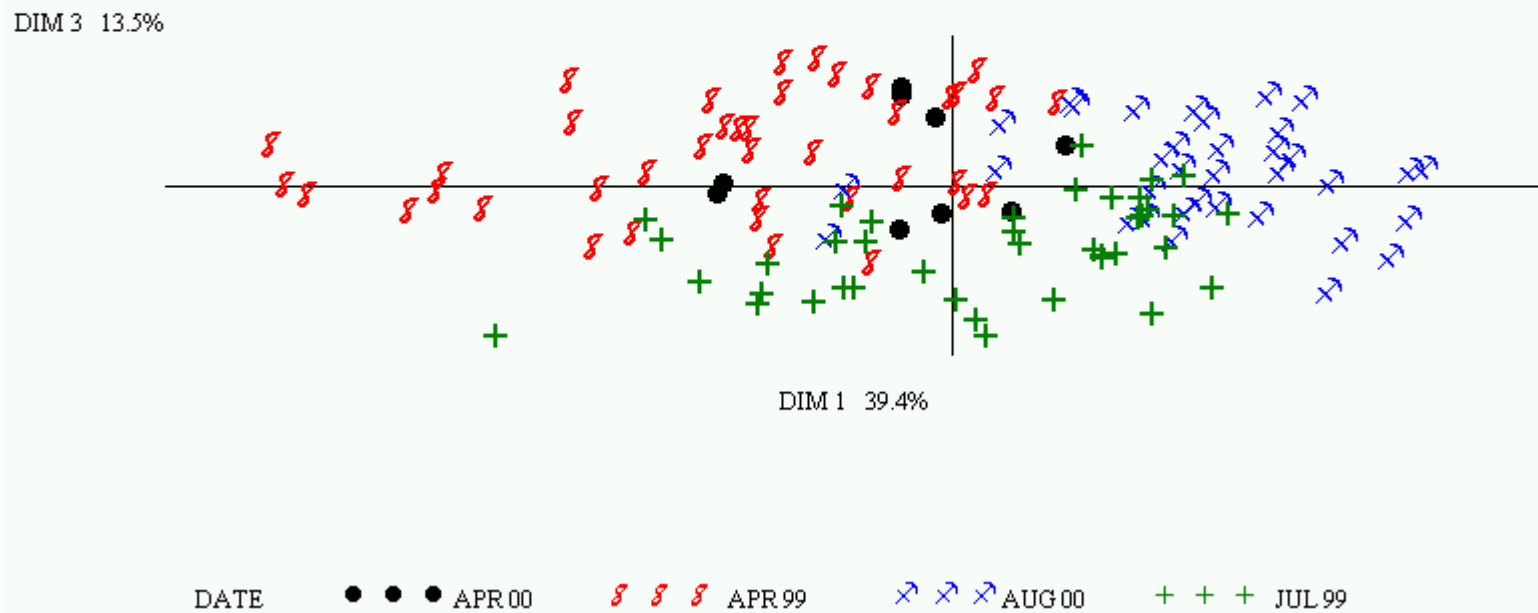


Figure 3: April 1999 Effect of cover crop on microbial communities in vineyard soils.

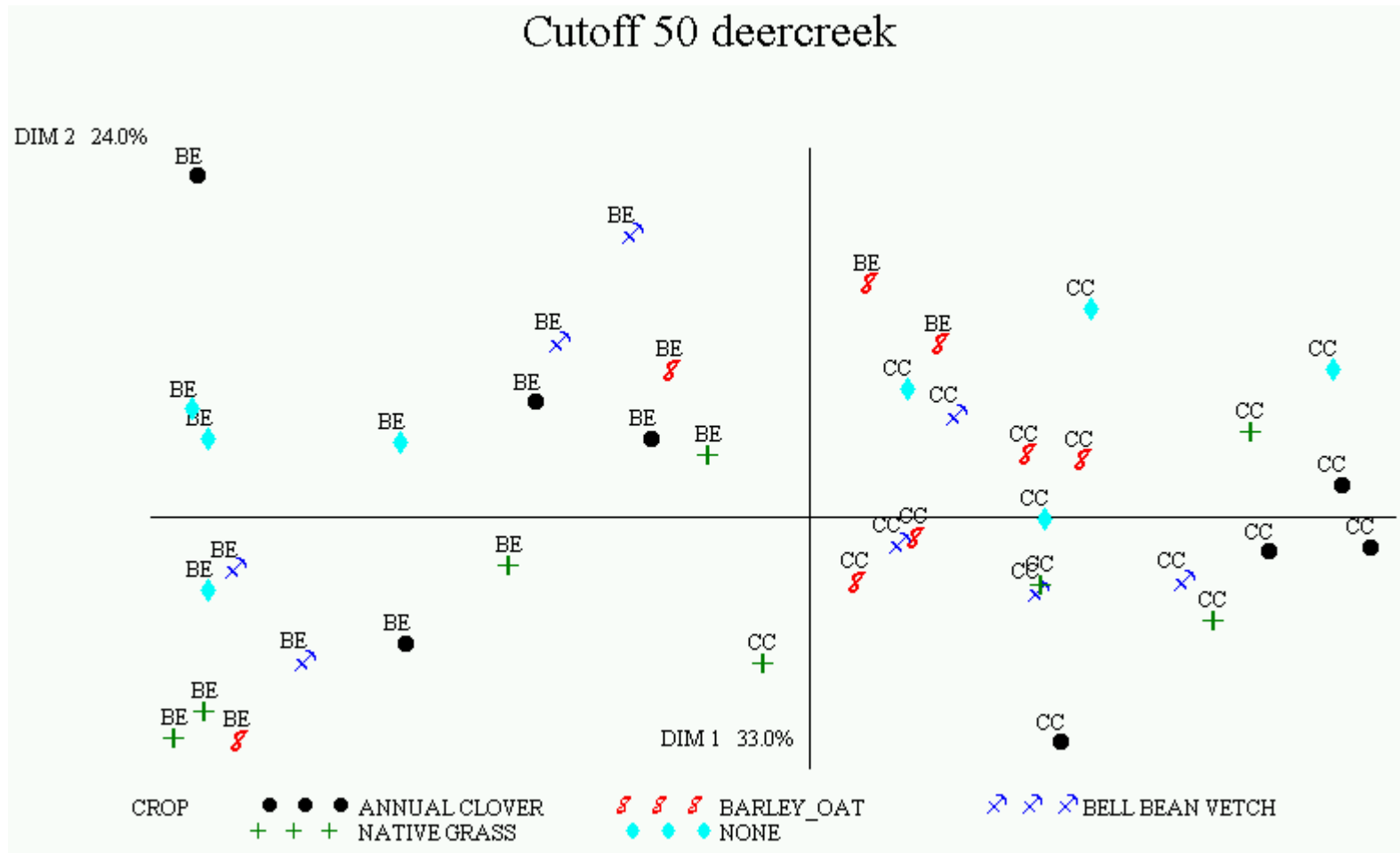


Figure 4: Lipid loadings associated with Fig.3

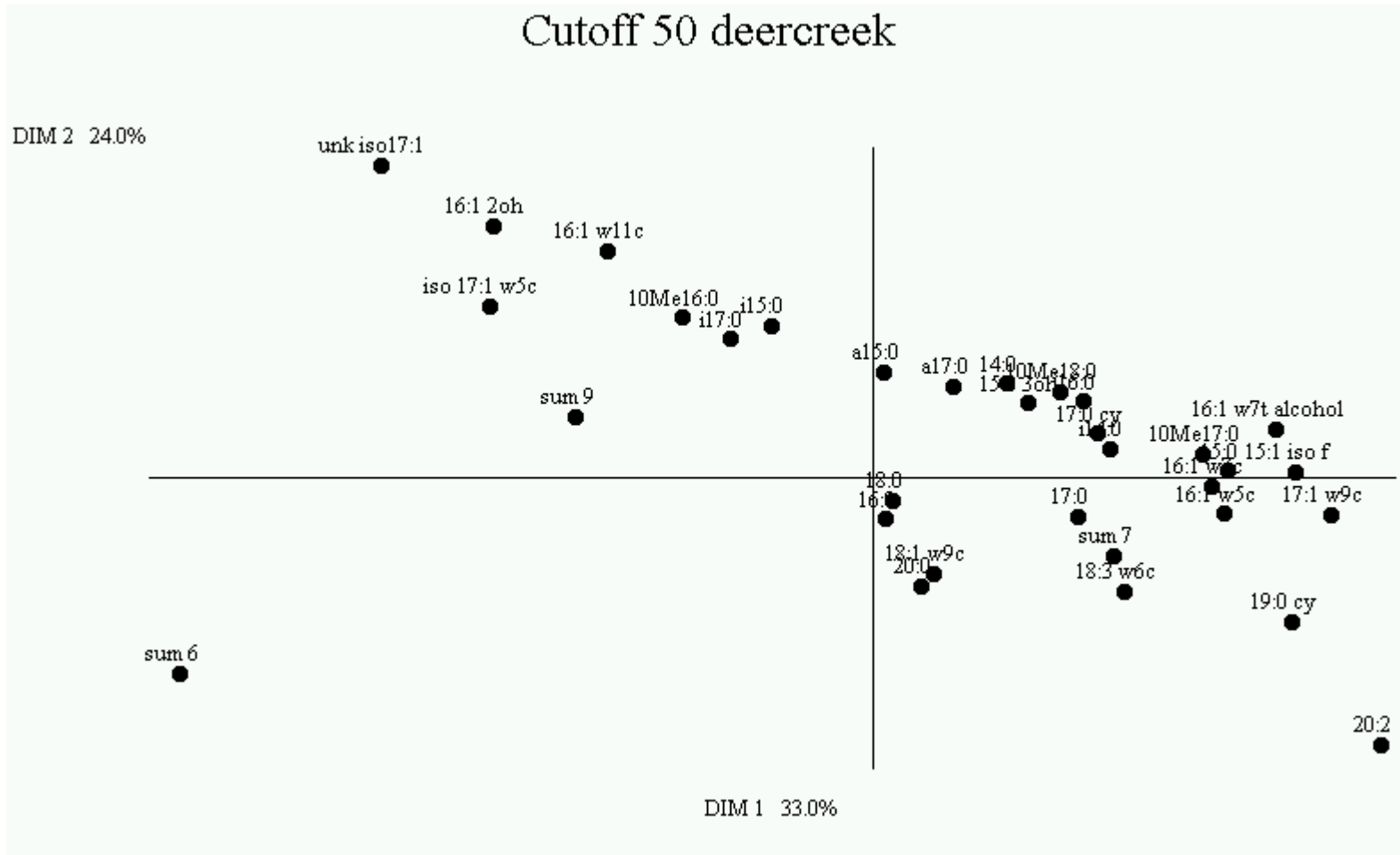


Figure 5: July 1999 Effect of cover crop on microbial communities in vineyard soils.

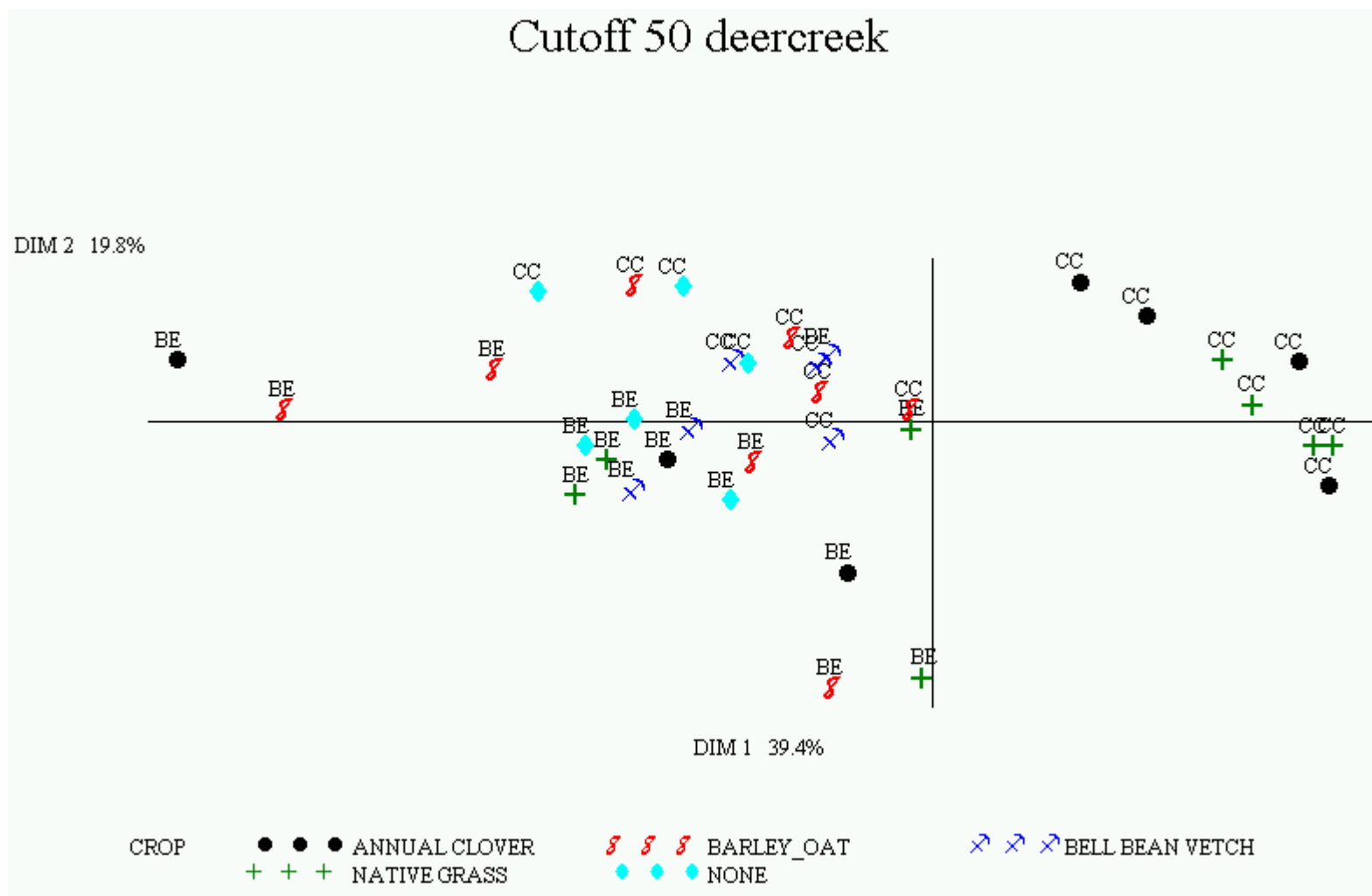


Figure 7: April 2000 Effect of cover crop on microbial communities in vineyard soils.

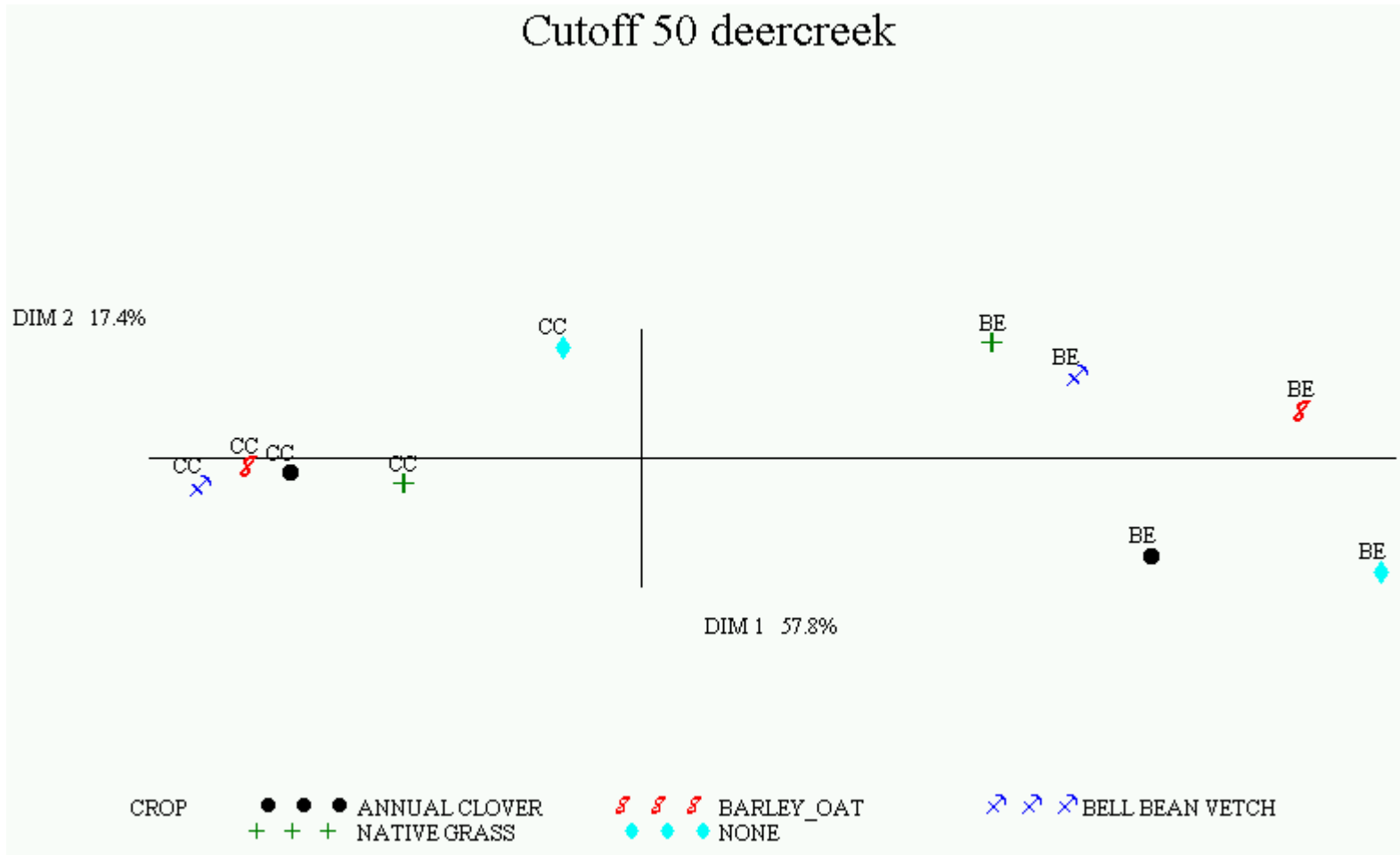


Figure 8: Lipid loadings associated with Fig.7

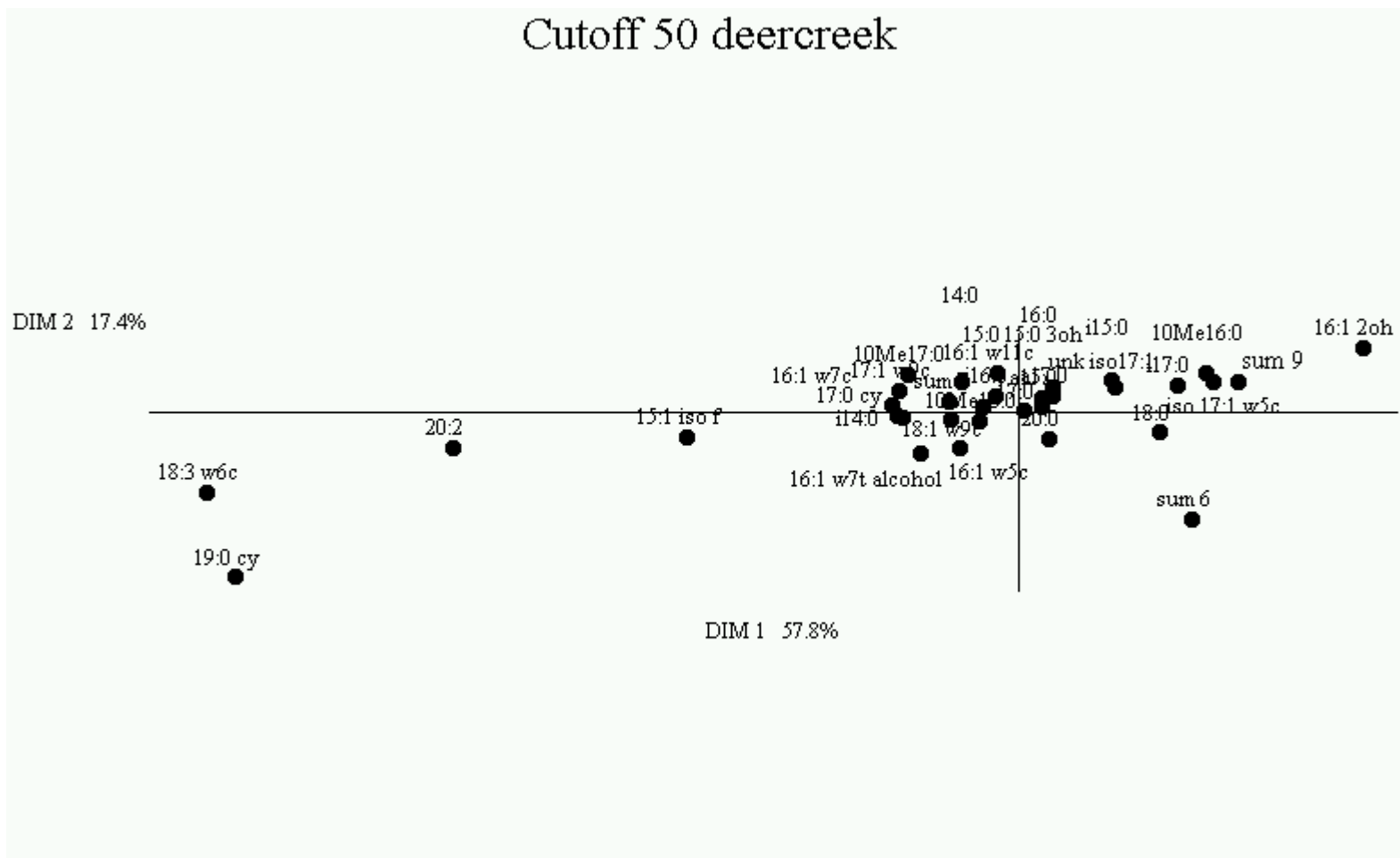


Figure 9: August 2000. Effect of cover crop on microbial communities in vineyard soils.

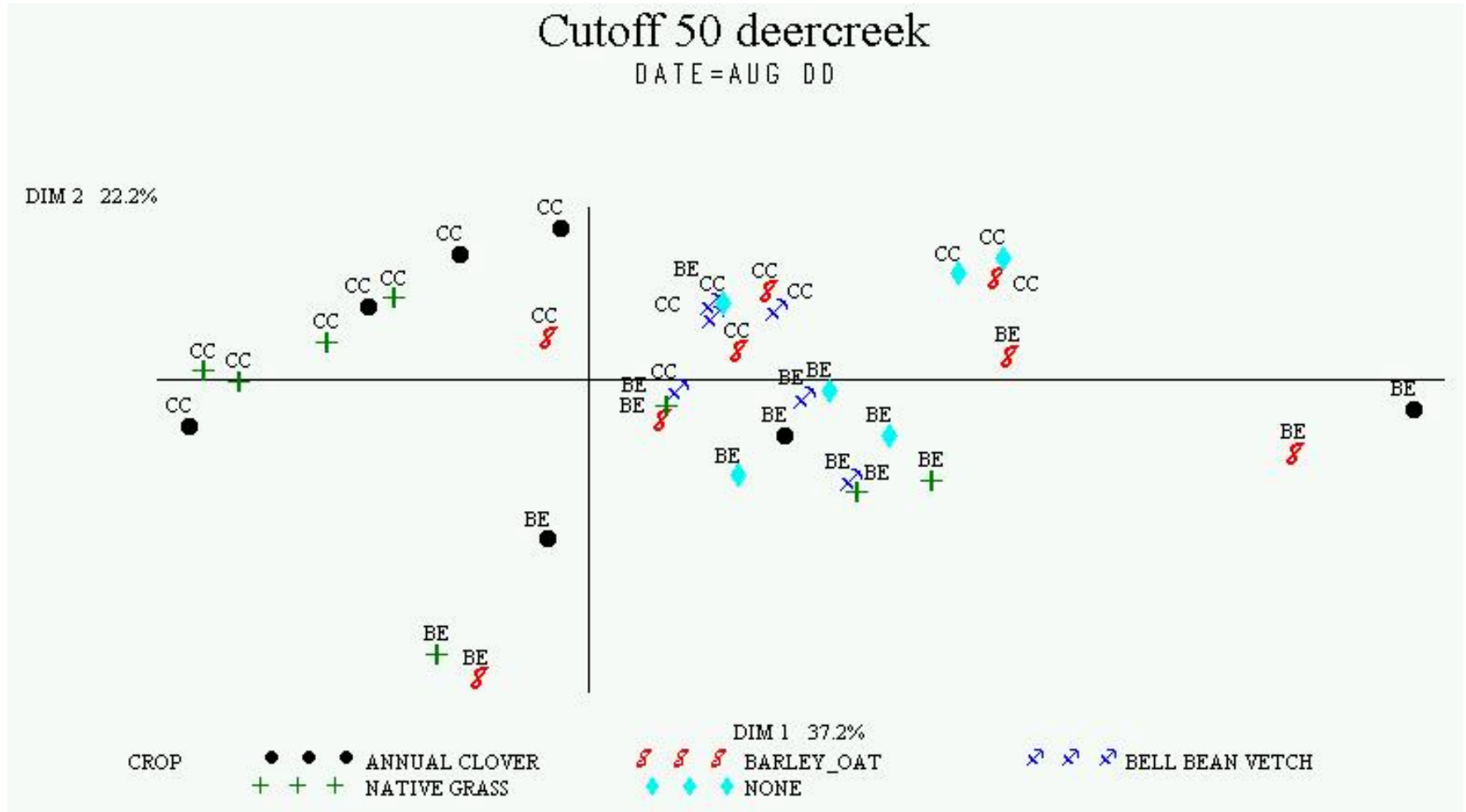


Figure 10: Lipid loadings associated with Fig.9

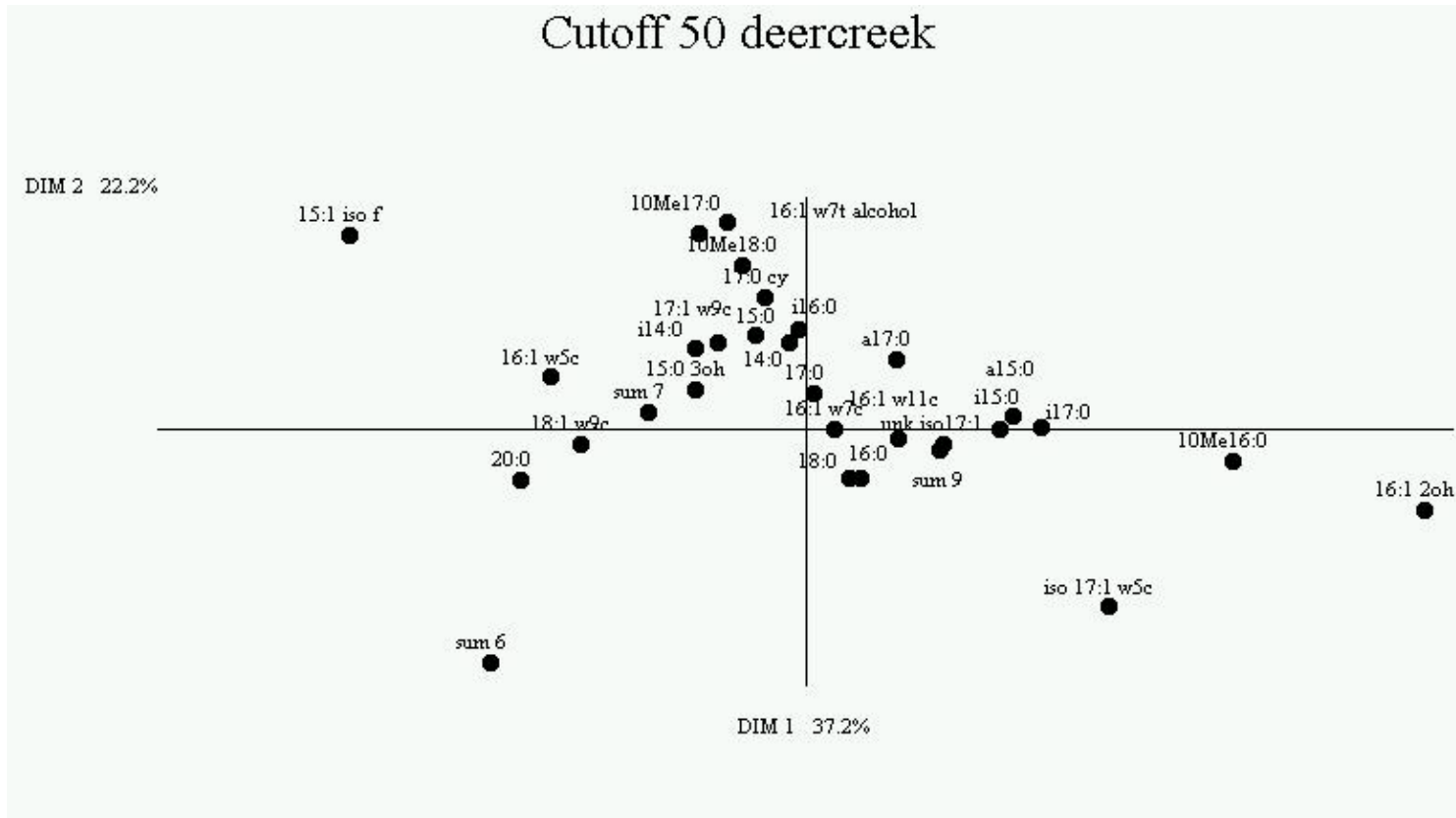


Table 1: Microbial Biomarker lipids by Cover Crop

(Average values in nanomoles/gram dry soil)

	Gram+ Bacteria <i>Branched</i> <i>% of total</i>	Aerobic Bacteria <i>Cyclopropyl</i> <i>% of total</i>	Fungi <i>18:2w6</i> <i>% of total</i>	Number of lipids	Total biomass <i>Total PLFA</i>
BERM					
ANNUAL CLOVER	25.37	3.16	7.92	36	19.9
BARLEY_OAT	25.58	3.13	7.10	34	16.3
BELL BEAN VETCH	25.03	3.07	6.85	34	16.6
NATIVE GRASS	24.97	3.08	7.65	36	21.0
NONE	25.38	3.13	7.99	35	18.2
COVER CROP					
ANNUAL CLOVER	23.61	4.16	5.02	37	26.0
BARLEY_OAT	26.59	3.87	5.42	35	20.2
BELL BEAN VETCH	24.49	4.07	5.49	37	23.2
NATIVE GRASS	24.86	3.66	5.75	37	28.0
NONE	26.56	3.95	4.59	35	18.0

Table 2: Biomarker trends in berm and cover crop regions over time

(Average values in nanomoles/gram dry soil)

	DATE	APR 99	st dev	JUL 99	st dev	APR 00	st dev	AUG 00	st dev
Total PLFA (relative biomass)	BE	15.60	6.78	24.74	5.20	13.80	3.66	15.41	5.17
	CC	21.96	8.01	22.93	4.01	22.64	4.90	24.68	11.05
18:2w6(% of total) (fungi)	BE	9.85	3.24	7.00	2.15	8.10	2.04	4.84	1.66
	CC	7.06	1.98	3.85	0.87	6.22	1.07	4.38	1.18
Branched(% of total) (bacteria)	BE	23.53	2.95	23.34	1.95	24.66	1.31	30.24	4.94
	CC	22.53	2.25	25.28	1.64	22.77	1.56	29.26	4.27
Cyclopropyl(% of total) (aerobic bacteria)	BE	02.66	0.36	3.40	0.53	2.92	0.13	3.38	0.45
	CC	03.14	0.33	4.46	0.41	3.72	0.39	4.39	0.41