Plant and Soil **206:** 151–161, 1999.

Fatty acid methyl ester (FAME) profiles as a tool to investigate community structure of two agricultural soils

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Received 24 November 1997. Accepted in revised form 1 October 1998

Key words: community structure, fatty acid methyl esters, rhizosphere, soil quality

Abstract

Soil microbiological parameters may be the earliest predictors of soil quality changes. Recently, molecular techniques such as fatty acid methyl ester (FAME) profiles have been used to characterize soil microbial communities. Fatty acid methyl ester (FAME) from whole soil may be derived from live cells, dead cells, humic materials, as well as plant and root exudates. Our objective was to verify differences in FAME profiles from two agricultural soils with different plants. Soil samples were collected from Ritzville and Palouse silt loams for fatty acid analysis. Soil samples from wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), pea (*Pisum sativum* L.), jointed goatgrass (*Aegilops cylindrica* L.) and downy brome (*Bromus tectorum* L.) rhizospheres were also collected for fatty acid analysis. Principal component analysis (PCA) of the two soils explained 42% of the variance on PC1, which accounted for Palouse soil. Ritzville soil accounted for 19% of the variance on PC2. Factor analysis showed that rhizosphere microbial communities from various plant species may differ depending on the plant species. Presence of Gram-positive bacteria as identified by a15:0, i15:0, a17:0 and i17:0 peaks were similar between rhizosphere and nonrhizosphere soils. Gram-negative bacteria characterized by short chain hydroxy acids (10:03OH and 12:03OH) as well as cyclopropane acids (cy17:0) were higher in rhizosphere soil than nonrhizosphere. This indicates a possible shift in the bacterial community to more Gram-negative bacteria and fewer Gram-positive bacteria in the rhizospheres of the plants species studied.

Introduction

Soil quality may be defined as the ability of soil to sustain biological functions and promote plant and animal health (Doran and Parkin, 1994). The biological component of the soil is responsible for carbon and nitrogen cycling, soil tilth and structure (Tisdall, 1991), organic matter transformation (Parkinson and Coleman, 1991), and other functions. However, a quick and reproducible method to assess soil quality is lacking. The traditional approach had been the use of several microbial methods to study soil quality (Jordan et al., 1995; Turco et al., 1994). Some of these methods are labor intensive and require culturing of microorganisms.

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Methods involving biochemical components as biomarkers can be used to assess soil quality and quantify the diversity of microorganisms in agricultural soils. Such methods include analysis of lipids, especially fatty acid methyl esters (FAMEs), which can be an effective method for distinguishing microbial communities from soil which differ in management practices (Klug and Tiedje, 1993). In a study of FAME profiles of soil samples taken from a conventionally tilled corn field, Cavigelli et al. (1995) found a high degree of reproducibility among soil samples taken from the same landscape. In another report Pankhurst (1997) used the MIDI systems to analyze FAME profiles of soils subjected to different agricultural management practices and found different FAME profiles indicating of management-induced changes in the composition of microbial communities.

bottle and caps crimped onto the sampler bottles. This method is thought to extract fatty acids from all cell components, including the plasma membrane, mitochondria, and lipid inclusions. All reagents used were of high-performance liquid chromatography grade.

Analysis of fatty acids

Fatty acid methyl esters were analyzed by gas chromatography carried out by an automated procedure developed by Microbial I. D. Inc, Newark, DE (Sherlock Microbial Identification System, 1996). The system used the MIS Eukary method designed to analyze fatty acids from cells in environmental samples. This is similar to the Aerobe or the clinical method except samples are run for 38 min, long enough for fatty acids with up to 28 carbon to pass through the column. The system consisted of a GC (HP5980)-Hewlett Packard, Wilmington, DE with a flame ionization detector, HP-IB communications, HP 3365 ChemStation software and computer. The HP 3365 ChemStation software operates the sampling, analysis and integration of the chromatographic sample. The temperature program was ramped from 170 °C to 250 °C at 5 °C per minute. Then the temperature was increased to 310 °C for 2 min to allow cleaning of the 25 m × 0.2 mm fused silica capillary column. Hydrogen was the carrier gas, nitrogen was the make up gas and air was used to support the flame at a flow rate of 30, 30, and 400 mL min^{-1}, respectively.

Statistical analysis

Data analysis was performed using SAS (SAS, 1988). Means and standard deviations for the individual fatty acids in triplicate sample FAME profiles were determined to compare area percent FAME of samples from the study areas. PCA was used to compare FAME profiles between the two soils and among rhizosphere and nonrhizosphere soils as well as show relationships among different samples with multiple variables (fatty acids) from each sample. Data were then presented as a 2D or 3D plot for better understanding of the relationship. PCA explains the variance-covariance structure through a few linear combination of the original variance (FAMEs) with coefficients equal to the eigenvectors of the correlation matrix (Jolliffe, 1986). To obtain a better and more elaborate separation of rhizosphere and nonrhizosphere samples, factor analysis was performed as an extension of PCA. Orthogonal transformation of the original data with rotated loading was used to compute factor scores that resulted in a simpler structure.

Results

FAME composition

The mean percent distribution of fatty acids from rhizosphere, nonrhizosphere, and greenhouse samples are shown in Tables 1 and 2. These include fatty acids with carbon length 10–20, since these are the dominant fatty acids in bacterial lipids (Haack et al., 1994). A total of forty-nine fatty acids from field samples and sixty-seven fatty acids from greenhouse samples with carbon length 10–20 were found. Palmitic acid (16:0) was a common fatty acid in all the samples with the highest area percent. In addition to palmitic acid, other common and abundant fatty acids extracted from field and greenhouse samples were 14:0, a15:0, i15:0, i16:0, 16:1ω7c, 18:0, 18:1ω9c, 18:2ω6c, and 18:3ω6c. These fatty acids were present in all the subsamples and made up about 55% and 37% of total fatty acid content in the field and greenhouse samples, respectively.

Monounsaturated fatty acids were found in the highest amount in both field and greenhouse samples, followed by saturated and branched chain fatty acids. Plant rhizospheres differed in the amount of OH groups. Wheat rhizospheres exhibited the highest amount and concentration of OH-FAMEs while downy brome rhizospheres showed the lowest amount. The i15:0, a15:0, i17:0 and a17:0 fatty acids commonly used as signature fatty acid for bacteria (Ratledge and Wilkinson, 1988) and 18:2ω6c for fungi (Federle, 1986) were present in all the samples. The polyunsaturated fatty acid biomarker 18:2ω6c of fungal origin accounted for 4.71–6.64 area percent FAME profile with the rhizosphere and nonrhizosphere samples showing significant higher area percent concentration of this biomarker than did the greenhouse samples. The concentration of this biomarker was higher in downy brome rhizospheres than in each of the other four plants.

Comparison of field with greenhouse rhizosphere samples (Table 3) showed that the ubiquitous fatty acid 16:0 was higher in all the field samples than greenhouse samples, and significantly higher in jointed goatgrass and wheat rhizospheres in the field than in the greenhouse. The a15:0, i15:0, i16:0, a17:0 generally used as signature fatty acids for Gram- positive

Table 2. Fatty acid composition from greenhouse samples

	Area percent fatty acid content (mean±SD)				
Fatty acid	Barley	Downy brome	Jointed goatgrass	Pea	Wheat
Hydroxyl			Area percent		
10:0 2OH	0.31±0.61	–	0.29±0.58	0.36±0.60	0.45±0.47
10:0 3OH	0.20±0.27	0.22±0.02	0.19±0.22	0.26±0.18	0.16±0.22
12:0 2OH	0.32±0.24	–	0.12±0.13	0.18±0.12	0.06±0.13
12:0 3OH	0.33±0.22	0.34±0.01	0.34±0.23	0.29±0.19	0.27±0.28
16:0 2OH	0.64±0.43	1.29±0.10	1.35±0.55	0.72±0.61	1.38±0.73
16:0 3OH	0.29±0.33	–	0.29±0.35	0.21±0.26	0.18±0.25
20:0 3OH	0.40±0.79	0.94±0.06	–	0.39±0.79	0.81±0.84
cy17:0	1.09±0.74	1.05±0.05	1.15±0.14	1.32±0.20	1.08±0.64
Monounsaturated					
16:1ω5c	3.31±0.44	2.79±0.11	3.12±0.49	3.18±0.55	3.41±0.69
16:1ω7c	5.76±0.22	4.32±0.12	4.49±1.08	5.63±0.79	5.50±1.46
16:1ω9c	0.15±0.31	–	0.14±0.29	–	0.40±0.56
17:1ω8c	1.45±0.32	1.26±0.01	1.56±0.30	1.68±0.35	1.46±0.21
18:1ω9c	6.51±0.79	5.46±0.36	5.47±0.85	6.15±1.88	5.22±0.63
18:1ω9t	2.50±0.19	2.20±0.21	2.27±0.21	2.50±0.49	2.47±0.24
Straight chain					
12:0	0.67±0.87	0.82±0.12	0.99±0.53	1.08±0.52	0.88±0.77
14:0	2.12±0.48	1.89±0.21	2.10±0.26	2.28±0.23	2.19±0.28
15:0	0.88±0.25	0.80±0.15	0.78±0.20	0.86±0.12	1.00±0.16
16:0	10.4±0.76	7.69±0.26	7.75±1.16	9.04±1.87	8.98±1.37
17:0	–	–	0.16±0.20	0.20±0.24	0.08±0.17
18:0	1.93±0.47	1.25±0.09	1.28±0.12	1.64±0.55	1.62±0.26
20:0	0.98±0.15	0.70±0.02	0.67±0.51	0.77±0.09	0.82±0.06
Branched chain					
a13:0	0.25±0.30	0.23±0.02	0.22±0.25	0.30±0.21	0.21±0.30
i14:0	0.74±0.09	0.62±0.21	0.68±0.12	0.78±0.07	0.75±0.15
a15:0	3.20±0.27	2.66±0.23	2.74±0.28	3.04±0.41	2.93±0.38
i15:0	3.14±0.25	2.61±0.20	2.67±0.43	3.09±0.43	2.98±0.39
i16:0	2.19±0.18	1.50±0.14	1.61±0.35	2.03±0.39	1.84±0.28
a17:0	0.99±0.11	0.91±0.11	0.93±0.12	0.98±0.09	0.97±0.31
i17:0	1.06±0.22	0.62±0.11	0.69±0.14	0.90±0.27	0.84±0.19
Polyunsaturated					
18:2ω6c	4.35±0.56	4.98±0.23	5.71±2.35	4.47±0.87	4.34±1.87
18:3ω6c	1.41±0.10	0.93±0.31	1.23±0.26	1.21±0.25	1.06±0.18

– Below detection limit by GC.

only variables with eigenvalue greater than one should be used for further analysis when correlation matrix is used in PCA. In our first analysis, we computed the correlation between principal components and fatty acids for PC1 and PC2. In PCA, the eigenvectors determine the directions of maximum variability and the eigenvalues specify the variances. Based on this information, 26 fatty acids with the highest loadings on the PCA were used to construct the bi-plot. We chose the 26 fatty acids with correlation $r \geq 0.55$ or -0.55 between variables (fatty acids) and the principle component scores. Results of this analysis showed two distinct clusters, with most of the Palouse soil clustering along PC1 and the Ritzville soil along PC2 (Figure 1). The percentage of variance explained by PC1 was 42%, while PC2 explained 19% of the variance, for a cumulative total of 61%. A screen plot analysis indicated that no further information would be gained by evaluating the third principal component (PC3 = 8%).

The loadings of fatty acids for PC1 separated Palouse soil, and these fatty acids were well-correlated ($r \geq 0.55$ or ≤ -0.55). These fatty acids include 10:03OH, 12:03OH, a13:0, i13:0, i15:0, 16:03OH, i16:0, cy17:0, 17:1ω3c, and 17:1ω6c. High variability in the microbial community of the Palouse soil was explained by PC1 which contributed 42% of the variance. Fewer fatty acids that separated Ritzville soil on PC2 had high loadings with correlation coefficient greater than $r = 0.55$, and these were 12:02OH, a15:0, 16:1ω6c, a17:0, and i17:0. The contribution of different fatty acids from different bacterial groups to PC1 and PC2 suggest that the two soils have different microbial composition.

Microbial community structure of rhizosphere and nonrhizosphere soils

Rhizosphere and nonrhizosphere fatty acid profiles were analyzed in a two-step process for community structure assessment. Data set exploration using PCA was used to suggest community structure and relationships of rhizosphere and nonrhizosphere FAMEs, and factor analysis for separation of FAMEs into different clusters with respect to the different plants. Data set exploration using PCA was accomplished by a bi-plot with all the fatty acids and then with a reduced number of fatty acids. The process explained above was used for the selection of fatty acids for the reduced data set. The bi-plot used 18 fatty acids that showed most of the nonrhizosphere samples clustering in the middle of the bi-plot (Figure 2). About 50% of the rhizosphere samples also clustered in this section of the plot while the other half was scattered above and to the left. It is shown from the bi-plot that rhizosphere samples were found in all sections. The spatial variability exhibited by the rhizosphere profiles suggest a more diverse population in the rhizosphere soil than in the nonrhizosphere soil.

When 18 fatty acids were used in the PCA, 51% of the total variation could be explained by the first two principal components. To elucidate differences of FAME profiles among rhizosphere and nonrhizosphere soil from different plants, factor analysis was used to obtain FAME profiles that had the greatest influence on the separation in Figure 2. Orthogonal transformation of the original data with rotated loading was used to compute factor scores. Figure 3 depicts a three dimensional plot using orthogonal transformation matrix with rotation method-Varimax.

The factor analysis model using 18 fatty acids separated the four plants into three clusters. Downy brome clustered in the middle (small ellipse) when projected onto the horizontal axis, barley and wheat clustered within the bottom group, and jointed goatgrass was on the top group (Figure 3). The clustering of fatty acid profiles in the bottom group from wheat and barley samples suggests that there may be different microbial communities among the rhizospheres of these plants compared to jointed goatgrass and downy brome.

Discussion

As shown in Figure 4, various plants had different fatty acid composition in their rhizospheres, with field rhizospheres showing the highest concentration of fatty acids. It should also be noted that high variability in fatty acid concentrations were found in the field samples than in the greenhouse samples. The differences in the proportion of different FAMEs within different plant rhizospheres gives a possible indication of the diverse group of microorganisms occupying the rhizosphere of these plants. The differences in area percent FAME profiles between the greenhouse and the field samples is a good indicator of differences in microbial community structure between the two environments (Table 3).

The presence of a large proportion of branched fatty acids, markers for Gram-positive bacteria, suggested high concentrations of this group of bacteria in soil samples. Some of the fatty acids reported to be of

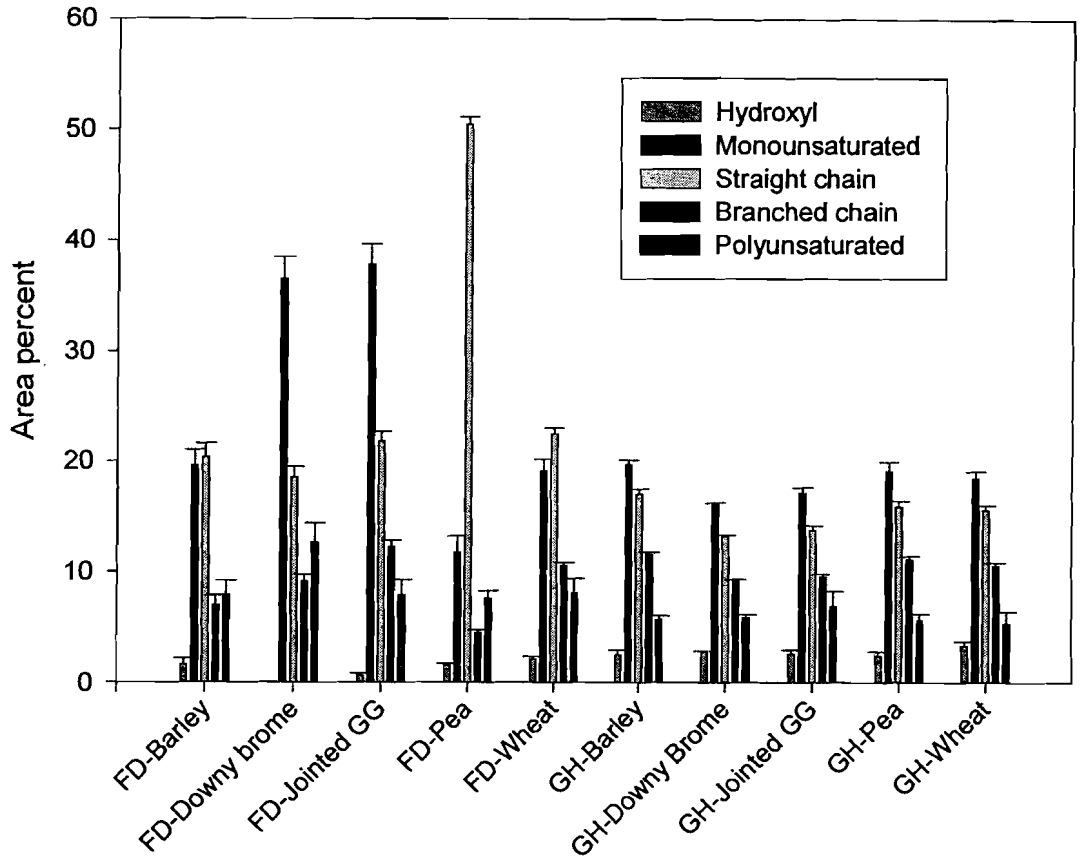

Figure 4. Average concentrations of FAME groups in the rhizospheres of different plants grown in the field and greenhouse. Results obtained by adding fatty acid concentrations from their respective groups from each plant used in the study. Bars represent standard errors of values averaged across replicates of each fatty acid.

acids from different taxonomic groups described in the literature.

Higher concentrations of cyclopropane fatty acid (cy17:0) were found in the rhizosphere of the crops studied, except downy brome. Higher amount of cy17:0 in the rhizosphere indicates probable increases in certain groups of Gram-negative bacteria, suggesting a shift in the community structure towards more Gram-negative bacteria in the rhizosphere than in the nonrhizosphere. This assessment was further supported by the high proportions of β-hydroxy fatty acid group found in the rhizosphere compared to nonrhizosphere. This isomer has been reported as constituent of Gram-negative bacteria (Wollenweber and Rietschel, 1990), especially the *Pseudomonas* species. In general, the cyclopropane fatty acids are usually produced by a number of Gram-negative bacteria such as *Cromatium, Legionella, Rhodospirillium* and *Campylobacter* (Wilkerson, 1988). However, Vesta and White (1989) had suggested that the cyclopropane fatty acids could be indicative of anaerobic conditions. Our results showed large amounts of these fatty acid in the rhizosphere community of our samples, supporting the findings of Tunlid et al. (1985) that reported large amounts of cyclopropane fatty acids from community level fatty acid profiles of rhizosphere bacterial communities.

The 18:2ω6c, commonly used as a fungal biomarker was significantly higher in most of the field than the greenhouse rhizospheres. The decrease in the area percent concentration of this fatty acid in the greenhouse may be due to physical disturbances of the soil and changes in temperature from about 20 °C in the field to the storage temperature of 4 °C in the lab for a period of 6 wk before they were used for the greenhouse experiment. Frostegård et al. (1996)

Pankhurst C E 1997 Biodiversity of soil organisms as an indicator of soil health. *In* Biological Indicators of Soil Health. Eds. C E Pankhurst, B M Doube and V V S R Gupta. pp. 297–324. New York, NY.

Parkinson D and Coleman D C 1991 Microbial communities, activities, and biomass. Agric. Ecosys. Environ. 34, 3–33.

Petersen S O and Klug M J 1994 Effects of sieving, storage, and incubation temperature on the phospholipid fatty acid profiles of a soil microbial community. Appl. Environ. Microbiol. 60, 2421–2430.

Ratledge C and Wilkinson S G 1988 Microbial lipids, vol. 1. Academic Press. Inc., New York.

SAS Institute 1988 Users guide: statistic version 6. Statistical Analytical Inst., Carey, NC.

Sherlock Microbial Identification System 1996 Operating Manual, version 6. Newark, Delaware.

Tisdall J M 1991 Fungal hyphae and structural stability of soil. Aust. J. Soil Res. 29, 729–743.

Tunlid A, Baird B H, Trexler M B, Olsson S, Findlay R H, Odham G and White D C 1985 Determination of ester-linked fatty acids and poly β-hydroxybutyrate for the estimation of bacterial biomass and activity in the rhizosphere of the rape plant *Brassica napus* L. Can.J. Microbiol. 31, 1113–1119.

Turco R F, Kennedy A C and Jawson M D 1994 Microbial indicators of soil quality. *In* Defining soil quality for a sustainable environment. Eds. J W Doran, D C Coleman, D F Bezdicek and B A Steward. pp. 73–90. SSSAJ special publ. 35, Madison, WI.

Vestal J R and White D C 1989. Lipid analysis in microbial ecology. Bioscience 39, 535–541.

Zelles L, Bai Q Y, Beck T and Beese F 1992 Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biol. Biochem. 24, 317–323.

Wilkinson, S G 1988. Gram-negative bacteria. *In* Microbial Lipids. Vol. 1. Eds. C Ratledge and S G Wilkinson. pp. 299–488. Academic Press, London.

Wollenweber H W and Rietschel E T 1990 Analysis of lipopolysaccharides (lipid A) fatty acids. J. Microbiol. Methods 11, 195–211.

Section editor: H Lambers

reported that incubation of forest soils seems to affect fungal biomass, resulting in a significant decrease in 18: 2ω6c. Also, Petersen and Klug (1994) showed a significant drop in this fatty acid and explained that filamentous fungi were more susceptible to damage done by sieving than single-celled organisms.

Community structure analysis by PCA

In our studies, PCA was used to reduce the dimensionality of our data and graphically show the separation of the two soils into two distinct clusters. Despite the difficulties in taxonomic differentiations of bacterial groups in the samples, PCA clearly showed differences between the two soils (Figure 1). This was in agreement with results obtained using phospholipid and Biolog GN analysis of the two soils (Ibekwe and Kennedy, 1998). The effects of climatic conditions and agricultural practices have resulted in these two soils from eastern Washington State having different microbial community structure. The clustering of 42% of the variances in PC1 which accounted for all the PC scores from Palouse soil suggest that Palouse soil has greater microbial variability than the Ritzville soil which contributed 19% of the variances mainly in PC2. This is in agreement with Zelles et al. (1992), using phospholipid fatty acids which showed that different management levels can be used to separate soils. Also, Pankhurst (1997) used the MIDI system to analyze FAME profiles of soils subjected to different agricultural management practices. In the study, PCA showed the soils to have different FAME profiles indicative of management-induced changes in the composition of microbial communities present.

We compared the results of our PCA to the PCA from Sherlock MIS, since we used the correlation matrix rather than the covariance matrix used by Sherlock MIS (data not shown). We used correlation matrix to standardize our data since the standard deviations of some of our individual FAMEs were large compared to the means. No differences in separation patterns were observed between the two approaches. Our results were in agreement with results from Sherlock MIS using the eukary method for MIS-FAME whole-soil-community profiles. It should be noted that the use of MIDI-FAME technique with whole soil communities is relatively new, compared to the wide acceptance in analyzing individual isolates using the aerobe method.

The Sherlock MIS system using the eukary method is a quick, inexpensive and reproducible method for describing microbial community structure in soils. By using FAME profiles in combination with PCA, we have shown that microbial community structures in two eastern Washington state soils are different. FAME data from different plant rhizospheres suggests that microbial community in the rhizosphere of most plants is influenced by the type of plant and environmental conditions. At present, we are using FAME as a tool in our laboratory to assess community changes under different cropping systems, and can foresee its use in future for soil quality assessment.

Acknowledgements

We gratefully acknowledge the assistance of Hui-Ling Lin for statistical analysis and Dr Alex Ogg for providing his research plot and plants used in this study. Support from USDA-ARS is greatly appreciated.

References

Bowen G D 1980 Misconceptions, concepts and approaches in rhizosphere biology. *In* Contemporary Microbial Ecology. Eds. D L Ellwood et al. pp. 283–304. Academic Press New York.

Cavigelli A C, Robertson P G and Klug M J 1995 Fatty acid methyl ester (FAME) profiles as measure of soil microbial community structure. Plant Soil. 170, 99–113.

Doran J W and Parkin T B 1994 Defining and assessing soil quality. *In* Defining Soil Quality for a Sustainable Environment. J W Doran, D C Coleman, D F Bezdicek and B A Steward (Eds). pp 3–21. SSSAJ special publ. 35, Madison, WI.

Federle T W 1986 Microbial distribution in soil-new techniques. *In* Perspective in Microbial Ecology. Eds. F Megusar and M Gantar. pp. 493-498. Slovene Society for Microbiology, Ljubljana.

Frostegård Å, Tunlid A and Bååth E 1996 Changes in microbial community structure during long-term incubation in soils experimentally contaminated with metals. Soil Biol. Biochem. 28, 55–63.

Haack S K, Garchow H, Odelson D L, Forney L J and Klug M J 1994 Accuracy, reproducibility,and interpretation of fatty acid methyl ester profiles of model bacterial communities. Appl. Environ. Microbiol. 60, 2483–2493.

Ibekwe A M, Kennedy A C 1998 Phospholipid fatty acid profiles and carbon utilization patternsfor analysis of microbial community structure under field and greenhouse conditions.FEMS Microbiol. Ecol. 26, 151–163.

Jolliffe I T 1986 Principal component analysis. Springer-Verlag.

Jordan D, Kremer R J, Bergfield W A, Kim K Y and Cacnio V N 1995 Evaluation of microbialmethods as potential indicators of soil quality in historical agricultural fields. Biol. Fertil. Soils 19, 297–302.

Kennedy A C and Smith K L 1995 Soil microbial diversity and the sustainabilityof agricultural soils. Plant Soil 170, 75–86.

Klug M J and Tiedje J M 1993 Response of microbial communities to changing environmentalconditions: chemical and physiological approaches. *In* Trends in Microbial Ecology. Eds. R Guerrero and C Pedros-Alio (eds). pp. 371–374. Spanish Society for Microbiology, Barcelona, Spain.

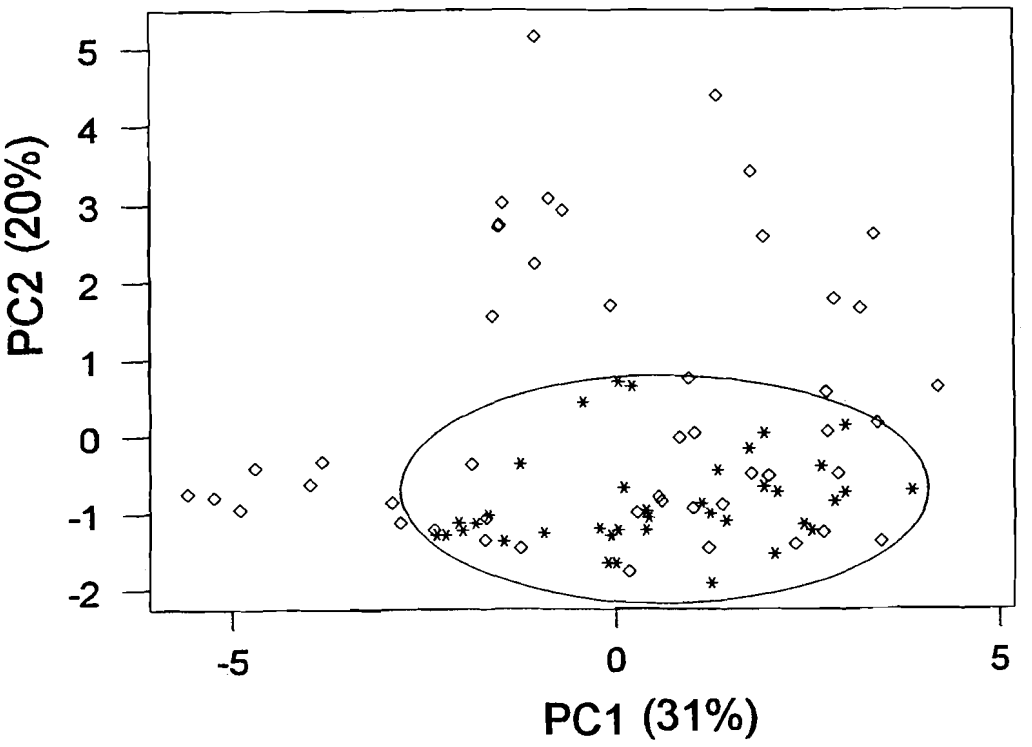

Figure 2. FAME profiles of * nonrhizosphere and ◊ rhizosphere soils. Eighteen fatty acids were used for this bi-plot.

Gram-positive origin (Ratledge and Wilkinson, 1988), a15:0, i15:0, i16:0, a17:0 and i17:0 were present in high proportion in both rhizosphere and nonrhizosphere soil samples. However, no differences were found in their area percent concentrations between the two environments. With the present knowledge of microbial community structure, it is difficult to assign taxonomic interpretations to community level fatty acid profiles in soil. We cannot say with certainty that a particular group of bacteria occupies the rhizosphere of certain plants or nonrhizosphere soil, however, qualitative conclusions can be drawn on community structure based on clustering of FAMEs in a particular environment compared to others.

Ratledge and Wilkinson (1988) suggested that the a15:0 and i15:0 are signature fatty acids for Gram-positive bacteria, such as *Clostridium/Bacillus* lines, but results by Haack et al. (1994) showed that some conclusions about community taxonomic compositions derived from analysis of community level fatty acid profiles may be based on inappropriate generalizations. Results by Tunlid et al. (1985) with rape plant (*Brassica napus* L.) rhizosphere showed high frequency of a15:0 and i15:0 from Gram-negative bacteria isolated from the rhizosphere of these plants. Our results showed wide variation in composition of fatty

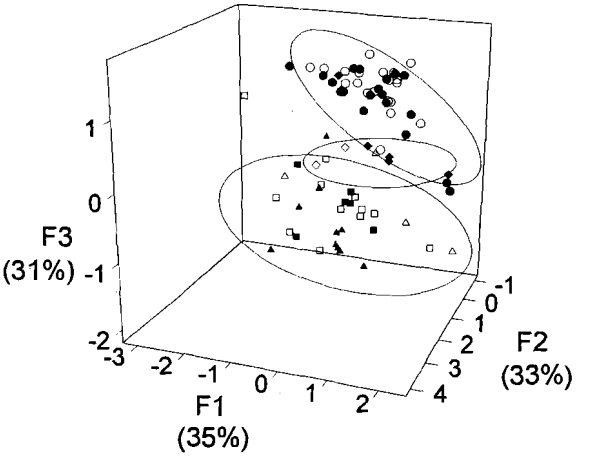

Figure 3. Factor analysis showing variation in FAME pattern in Palouse soil planted with different crops. Scores are for eighteen FAME profiles from jointed goatgrass (●) rhizosphere, (○) nonrhizosphere; wheat (▲) rhizosphere, (△) nonrhizosphere; barley (B) rhizosphere, (E) nonrhizosphere; downy brome (♦) rhizosphere, (◊) nonrhizosphere.

Table 3. Comparison of area percent concentrations of selected fatty acids in rhizosphere between greenhouse and field samples

Fatty acids	Barley		Jointed goatgrass		Pea		Wheat	
	Greenhouse	Field	Greenhouse	Field	Greenhouse	Field	Greenhouse	Field
	Area percent							
16:1 w7c	5.76	5.27	4.49	10.63*	5.63	3.39*	5.50	6.86
18:1 w9c	6.51	6.76	5.49	10.03*	6.15	7.68	5.22	8.06*
14:0	2.12	4.06*	2.10	2.39	2.28	36.14*	2.19	4.23*
15:0	0.88	0.07*	0.78	0.56	0.86	0.34	1.00	0.90
16:0	10.40	11.90	7.85	14.72*	9.04	7.69	8.98	12.75*
18:0	1.93	0.95	1.28	2.80*	1.64	2.76	1.62	1.63
a15:0	3.20	2.75	2.74	3.66*	3.04	1.71	2.93	3.05
i15:0	3.14	2.66	2.67	3.54*	3.09	1.34	2.98	2.78
i16:0	2.19	1.35	1.61	2.79*	2.03	1.00	1.84	2.12
a17:0	0.99	0.26*	0.93	1.13	0.98	0.47	0.97	1.16
18:2 w6c	4.35	5.80*	5.71	5.95	4.47	6.16*	4.34	6.41*
18:3 w6c	1.41	2.14	1.23	2.00*	1.21	1.43	1.06	1.71

* = Significant differences using student *t*-test.

bacteria were higher in jointed goatgrass field samples than in the greenhouse samples. However, pea plant had higher concentrations of these fatty acids in the greenhouse rhizospheres than in the field. There were no differences of these fatty acids in barley and wheat between the two environments. These results suggest that growth rate of microorganisms in the rhizosphere depends on plant species and the environment. Variations in the amount of fatty acids between the two environments may also be due to the age of plants at the time of sampling. Bowen et al. (1980) reported that growth of one *Pseudomonas* sp. in the rhizosphere of *Eucalyptus globulus* varies with species of this plant. In general, greater differences were observed between the rhizosphere of jointed goatgrass and pea in the two environments than in the other plants used in this study.

Principal component analysis of agricultural soils

To determine differences in microbial community structure between Ritzville and Palouse soils as well as among crop rhizospheres and nonrhizospheres, two approaches were used. Principal component and factor analyses were conducted to see if there was separation of FAME profiles between the two soils and among crops. PCA was used to view the discrimination of microbial community structure between Palouse and Ritzville soils. PCA was conducted with forty-four fatty acids that were present in the samples. This explained only 35% of the total variance in the first two

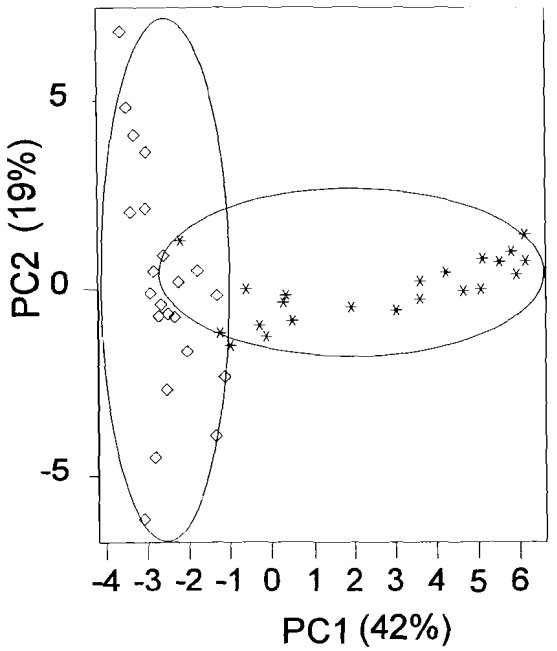

Figure 1. FAME profiles of two soils from Eastern Washington State. * Palouse soil (mostly on PC1 with 42% of the variance), ◊ Ritzville soil (mostly on PC2 with 19% of the variance). Twenty six fatty acids were usedfor the bi-plot.

dimensions. Due to the low amount of variance explained by the first two dimensions of the PCA, we ran PCA on matrices with fewer fatty acids. The initial approach used the Kaiser's rule (Jolliffe, 1986), that

To measure community structure in agricultural soils, *in situ* assessment of microbial diversity is needed. Previous studies (Kennedy and Smith, 1995), using the same soil as in this study showed a shift in community structure between the grass prairie and cultivated field when biomass C, phosphatase, and substrate utilization were measured. The objectives of this study were to evaluate whether there are differences in soil microbial community structure based on FAME profiles between the two agricultural soils and to assess differences in community structure in the rhizospheres of different plant species within a landscape

Materials and methods

Soil sampling and site description

Soil samples for the study were collected from the Lind Research Station, Lind, WA, and from the USDA Palouse Conservation Field Station, Pullman, WA in late spring 1996. The soil at Lind is a Ritzville silt loam (coarse-silty, mixed, mesic Calciorthidic Haploxeroll), and at Pullman is a Palouse silt loam (Pachic Ultic Haploxeroll). A total of 20 soil samples from an uncultivated grassland were collected over a 0.5 ha area from the Lind site on a transect of 5 samples each. The Lind site has an annual precipitation of 230–300 mm with coarse soil very susceptible to wind erosion. The usual cropping system on the site is winter wheat-spring barley or wheat-fallow rotation with some conventional tillage. The Palouse site has an annual precipitation of about 450–600 mm and the main crops grown on the site include wheat, barley, peas, lentils (*Lens culinaris*), and alfalfa (*Medicago sativa*). Conventional tillage and weed management are practiced at the Pullman site. Rhizosphere and nonrhizosphere samples were collected from the Palouse site over a 1.0 ha area. Five samples from each plant species were collected from each transect. For each plant species, a total of 10 rhizosphere and 10 nonrhizosphere samples were collected. All nonrhizosphere samples were collected less than 1 m from rhizosphere samples. The plot has a 15% slope. Wheat, barley, and pea rhizosphere and nonrhizosphere samples were collected at the bottom of the slope, while jointed goatgrass (*Aegilops cylindrica* L.) and downy brome (*Bromus tectorum* L.) samples were collected at the top of the slope separated by a grass walkway.

Rhizosphere soils were collected by pulling plants from the soil and shaking soil off from plants. Roots were cut from plants and put inside 250 mL tube and placed in dry ice. In the lab, the tubes were vigorously shaken and soil that was still held to the roots was collected and placed in ziplock plastic bags and immediately placed at −70 ° C until use. Nonrhizosphere soils (0-10 cm) were collected by using a 10 cm diameter stainless steel auger and treated as above. The soil auger was rinsed with diluted methanol between sampling to avoid lipid contamination.

Greenhouse study

Soil samples (100 g) from the two sites were placed in a 24.7×4.0 cm top diameter container. Seeds of the test plant species were placed on soil surface and 5 g soil added to cover seeds. Soils used for the study were maintained at field capacity. Plants were arranged in a complete randomized design with three replications and grown in the greenhouse for 21 d. Plants were watered as needed. At harvest, plants were removed from the containers and soil attached to the roots were maintained as above for lipid analysis. Soil samples for lipid analysis were passed through a 4-mm mesh screen and stored at 4 °C until ready to be used.

Extraction of fatty acid

Soil samples (1 g) were placed in Teflon-lined screw cap culture tubes (13×100 mm) and processed according to Sherlock Microbial Identification System (Sherlock Microbial Identification System, 1996) standard protocol. Briefly, FAME extraction from soil used a four-step procedure. (i) Saponification-lysis solution was added to soil to liberate the fatty acid from the cellular lipids by adding 1 mL of NaOH in aqueous methanol (45 g of NaOH in 150 mL methanol) and heating the subsamples in a 100 °C water bath for 30 min then cooling in a room-temperature water bath. (ii) Methylation-formation of methyl esters of the fatty acid by adding 2 mL of HCl in aqueous methanol (6.0 N HCl: 325 mL HCl in 275 mL methanol) and placing in an 80 °C water bath for 10 min and immediately cooled to room temperature. (iii) Extraction-transfer of the FAMEs from the aqueous phase to an organic phase in a 1 mL 1:1 (vol/vol) hexane:methyl tert-butyl ether (MTBE) and rotating subsamples end-over-end for 10 min. (iv) Base wash-aqueous wash of the organic extract prior to chromatographic analysis with 3 mL of 1.2% NaOH in H_2O by rotating the tubes end-over-end for 5 min. Finally, the organic phase containing the fatty acid methyl esters was removed from the tubes and placed in gas chromatography (GC) sampler