

Phospholipid fatty acid profiles and carbon utilization patterns for analysis of microbial community structure under field and greenhouse conditions

Abasiofiok M. Ibekwe *, Ann. C. Kennedy

USDA-ARS, Washington State University, Pullman, WA 99164-6421, USA

Received 11 November 1997; revised 30 March 1998; accepted 1 April 1998

Abstract

The description of soil microbial community structure by phospholipid fatty acid (PLFA) profiles is based on the relationship between the phylogeny of microorganisms and their PLFA profiles. Based on this relationship, two community based microbiological measurements, namely, potential C source utilization patterns in Biolog microtiter plates and PLFA profiles were used to examine metabolic fingerprints of soil microbial communities and changes in species composition between field and greenhouse soils. Field and greenhouse experiments were conducted using Palouse and Ritzville silt loams. Soil sampled under wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), pea (*Pisum sativa* L.), jointed goatgrass (*Aegilops cylindrica* L.), downy brome (*Bromus tectorum* L.), and control soils (no plants) were used for these analyses. Principal component analysis (PCA) of PLFA profiles and C source utilization patterns were used to describe changes in microbial biomass and metabolic fingerprints from the two soil types under field and greenhouse conditions. Biomass measurements from extractable PLFA profiles per g dry weight ranged from 28.8 nmol in wheat soil in the greenhouse to 71.4 nmol in pea soil in the field. In general, biomass was higher in all the field samples than in greenhouse samples. Principal component analysis of the two soils with different plants in the field and greenhouse showed clear separation. Principal component analysis of C utilization patterns on the effects of environment on soil microbial community yielded similar results with PLFA measurements. However, higher variability observed among different plants with the Biolog data resulted in the low amount of variance for Biolog data explained by the first two dimensions of the PCA. This suggests that PLFA may be more sensitive for community analysis than the Biolog technique. © 1998 Published by Elsevier Science B.V. All rights reserved.

Keywords: Phospholipid fatty acid; Carbon source utilization; Biolog; Metabolic fingerprint; Microbial community structure; Microbial biomass; Principal component analysis

1. Introduction

The quantitative description of microbial communities is one of the most difficult tasks facing mi-

crobial ecologists as the classical techniques of viable counting often recover only a small proportion of total cells [1]. At present, only two methods have overcome the problem of selective culturing while providing an unbiased view of the structure of complex microbial communities. These are analysis of microbial populations using ribosomal RNA

* Corresponding author. Tel.: +1 (509) 335-3662; Fax: +1 (509) 335-3842; E-mail: ibekwe@mail.wsu.edu

(rDNA) and phospholipid fatty acid analysis [2]. Microbial community analysis using rRNA (rDNA) can detect and identify community members with high specificity to the species and strain levels, as well as detect and suggest phylogenetic affinities of uncultured organisms [3]. However, these procedures are labor intensive and the time required for DNA extraction, screening and sequencing can be prohibitive. The alternative is the use of phospholipid fatty acids to quantify community structure without relying upon cultivation of microorganisms. This approach does not have the capability to identify microorganisms to species and strain level, but rather produces descriptions of microbial communities based on functional groupings of fatty acid profiles.

By using the different functional groupings from phospholipid fatty acids, White and Findlay [4] developed a community-level approach to characterize microbial community structure by evaluating shifts in phospholipid fatty acids from environmental samples. This approach is based on the assumptions that phospholipids make up a relatively constant proportion of the cell biomass and that fatty acid variation among taxonomic groups results in markers which can be used to interpret community-level profiles. Different groups of bacteria are characterized by specific phospholipid fatty acid profiles therefore, a change in phospholipid pattern in soil would indicate a change in microbial composition of that soil. This concept has resulted in the identification and quantification of viable biomass and community structure in sediments [5–7] and in agricultural soils [8,9].

Garland and Mills [10] adapted the Biolog redox technology based on community-level carbon source utilization patterns to characterize and classify microbial communities from environmental (soil, aquatic, and rhizosphere) samples. These authors used multivariate statistical methods to show distinctions in community structure among different environmental samples based on their carbon source metabolism. In a comparative study of natural bacterial communities (rhizosphere soil) and of an aerobic bioreactor, Garland and Mills [11] showed that substrate utilization patterns can be used as an indicator of community structure. In a more recent study, Garland [12] analyzed patterns of potential C source utilization by microbial communities as a means of

differentiating among and within rhizosphere samples of different crops. The author concluded that distinctive patterns of C source utilization were found for each crop type, and the differences among crops were consistent over a two year period. Zak et al. [13] demonstrated that the Biolog system can detect considerable variation in the ability of microbial communities to metabolize different C compounds, and found considerable differences among the communities of six ecosystems. Separation of a model microbial community was accomplished by principal component analysis (PCA) based on substrate oxidation. In a study to evaluate the environmental impact of hydrocarbon pollution in soil, characteristic shifts of the substrate utilization patterns, as tested with the Biolog system were observed with changes in hydrocarbon content of the soils [14].

The above two methods hold considerable promise as rapid methods for characterizing microbial communities. However, Haack et al. [15] expressed concern that the use of PCA on the degree of substrate oxidation is more problematic because: (i) the degree of substrate oxidation may be a function of inoculation density, (ii) different microorganisms which use the same substrate oxidize those substrates to different extents, and (iii) the substrate oxidation profiles of communities are not simple summations of the individual profiles of their constituent members. Wünsche et al. [14], expressed the same concern that patterns measured by the Biolog system reflect the metabolic activities of only a certain proportion of the community. Garland [12] dealt with some of these problems by using the average well color development (AWCD) = 0.75 (abs) as the best data point for analysis by PCA. This author suggested that PCA is more vulnerable to distortion due to outliers and sample errors. He suggested however, that detrended corresponding analysis (DCA) may minimize the effects of variations in AWCD, probably due to its weighted-averages transformation of the data prior to analysis.

Our objective is to use two microbiological measurements, phospholipid fatty acid profiles and C source utilization patterns, to characterize and identify microbial community structure between two agricultural soils under field and greenhouse conditions. The basic principles underlying the community-level analysis are better understood for the PLFA

analysis than the substrate utilization assay. The speed of substrate utilization by Biolog microtiter plates to characterize microbial communities can provide useful results if data are handled properly. Ultimately, we wish to use PLFA to produce a quantitative estimate of soil microbial biomass for the two soils from both the field and greenhouse. Results of PLFA from whole soil communities will be compared to that of plated communities to show whether plated communities can be used to describe community structure.

2. Materials and methods

2.1. Sampling site

Soil samples for the study were collected from the Washington State University Lind Research Station, Lind, WA, and from the USDA Palouse Conservation Field Station, Pullman, WA. The soil at Lind is classified as a Ritzville silt loam (coarse-silty, mixed, mesic Calciorthidic Haploxeroll), and at Pullman is classified as a Palouse silt loam (Pachic Ultic Haploxeroll). A total of 20 soil samples from wheat and fallow plots were collected over a 0.5 ha area from the Lind site on a transect of five samples each. The Lind site has an annual precipitation of 230 to 300 mm with soils which are very susceptible to wind erosion. The usual cropping system on the site is winter wheat-spring barley or winter wheat-fallow with tillage. The Palouse site has an annual precipitation of about 450 to 600 mm and the crops grown on the site include wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), pea (*Pisum sativa* L.), lentils (*Lens culinaris*), and alfalfa (*Medicago sativa*). Conventional tillage and weed management are practised at the Pullman site. Soil samples were collected from the Palouse site over a 1.0 ha area. Five soil samples were collected under each plant species from a transect of 20 m long, and control samples without plant influence were also collected. Only three samples from each soil under the plants used in this study were used for lipid analysis. Wheat, barley, and pea 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. Soils were collected by pulling plants from the soil and shaking the loosely held soil into plastic bags and immediately placing the bags on dry ice. Control soils were sampled to a depth of 7 cm by using a 10 cm diameter stainless steel auger washed with diluted methanol between sampling to avoid lipid contamination. Samples were stored at -70°C until lipid analysis.

2.2. Greenhouse study

The greenhouse study was conducted to compare the microbial community structure and biomass of soils under greenhouse conditions to those of field samples. Soil samples (100 g) were placed in a 24.7 cm long \times 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 days. Plants were watered as needed. At harvest, plants were removed from the containers and soil attached to the roots was maintained as above for lipid analysis and patterns of community sole carbon source utilization.

2.3. Substrate utilization patterns of microbial populations using the Biolog system

Biolog Gram-negative (GN) microtiter plates were used to analyze microbial community structure through substrate utilization patterns by soil microorganisms. Biolog (GN) plates contain 95 separate carbon sources and a blank well with no substrate. The redox dye tetrazolium contained in each well is reduced by NADH produced by respiratory pathways. The rate of color development depends upon the rate of respiration in the wells. In order to obtain substrate utilization patterns of whole soil communities, the cell suspensions were prepared by extracting the soils with phosphate buffer solution, serially diluting and adding 150 μl suspension of 10^{-3} dilution into the Biolog plates with an 8-channel repeating pipette. After incubation of microtiter plates at 25°C , absorbance data were recorded at 405 nm at 0, 24, 48 and 72 h with a Bio-Rad Plate Reader (Bio-Rad Lab, Richmond, CA). The 405 nm absorbance

was chosen to produce a conservative reading of activities [13].

2.4. Phospholipid extraction

Soil samples (2 g) were extracted using the modified method of Bligh and Dyer as described by Petersen and Klug [16]. Phosphate buffer (50 mM; pH 7.4) was added to give a total volume of 2 ml, and then 7.5 ml of 2:1 methanol-dichloromethane (DCM) was added. Samples were extracted by a Whirly mixer for 2 h, and then 2.5 ml of DCM and 10 ml of supersaturated NaBr solution (800 g L⁻¹) were added and mixed overnight on the Whirly mixer for phase separation. Samples were centrifuged at 7500 × *g* for 30 min and the lipid containing organic (top) phase was transferred to 10 ml Pyrex tubes with Teflon-lined caps and allowed to evaporate completely under a stream of nitrogen.

Phospholipid fatty acids were also extracted from culturable microorganisms in subsample soils to compare community structure between plated and whole soil community. Soil samples (1 g) were serially diluted in phosphate buffer (pH 7.2) and 1 ml from each 10⁻² dilution tube was transferred to a 150 × 10 mm petri dish containing trypticase soy broth agar (TSBA). Plates were incubated at 25°C for 36 h and stored at 4°C for 24 h before processing. The entire plated community was scraped into a 25 ml tube and PLFA extracted as above.

2.4.1. Separation of phospholipids

Phospholipids were separated by solid phase extraction. The dried lipid material was dissolved in 100 µl chloroform and separated on prepacked columns which selectively retained phospholipids, while non-polar lipids (neutral lipids and glycolipids) are eluted with solvents of lower polarity. Twelve columns were mounted on a vacuum manifold and connected to a pump via a side arm flask for collection of solvents. The columns were conditioned by sequentially adding 1.5 ml hexane (twice) and 1.5 ml hexane/chloroform (1:1). Vacuum was applied to the columns after the addition of each set of solvents. Dried lipid extracts were eluted with 100 µl of chloroform (3 times), and drawn through the columns. The chloroform was pulled through, and the columns were washed with 1.5 ml chloroform/2-

propanol (2:1) and 1.5 ml 2% acetic acid in diethyl ether to remove lipids of low and intermediate polarities. Clean pyrex tubes were placed under the columns to collect phospholipids which were eluted with 1 ml of methanol (twice). Methanol collected was allowed to evaporate under nitrogen before extraction of PLFAs. A mild alkaline methanolysis was carried out on the phospholipid fraction of the lipid to release and methylate the PLFA [17].

2.4.2. Analysis of PLFAs

Fatty acids were analyzed by gas chromatography carried out by an automated procedure [18] developed by Microbial I.D. Inc, Newark, DE. The system used the MIS Eukary method designed to analyze fatty acids from cells in environmental samples. Samples were run for 38 min, long enough for fatty acids with up to 28 carbons to pass through the column. The system consisted of a GC (HP5980) with a flame ionization detector, HP-IB communications, HP3365 ChemStation software and computer (Hewlett Packard, Wilmington, DE). The HP3365 ChemStation software operates the sampling, analysis and integration of the chromatographic sample. The temperature program ramps from 170°C to 250°C at 5°C per min. Then the temperature increases 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.

2.5. Statistical analysis

Data analysis was performed using SAS [19]. Means and standard deviations for the individual fatty acids in triplicate sample PLFA profiles were determined to compare percent total PLFA of samples from the study areas. PCA was used to compare PLFA profiles between the two soils and data were presented as a 3-D plot for better understanding of the relationship. We used Kaiser's rule [20], that only variables with an 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, PC2 and PC3. In PCA, the eigenvectors determine the directions of maximum variability and the eigenvalues specify the variances.

Table 1

Phospholipid fatty acid contents of soils under five plants and a control soil from field and greenhouse Palouse soil

| PLFA ^a | Percent total PLFA composition (mean ± S.D.) ^b | | | | | | | | | | | |
|-------------------|---|------------|-------------|------------|-------------------|------------|------------|------------|------------|------------|------------|------------|
| | Barley | | Downy brome | | Jointed goatgrass | | Pea | | Wheat | | Control | |
| | Field | GH | Field | GH | Field | GH | Field | GH | Field | GH | Field | GH |
| 16:0 | 2.51 ± 0.1 | 2.25 ± 0.3 | – | – | – | – | 2.53 ± 0.5 | 5.62 ± 1.3 | 2.19 ± 1.5 | 3.51 ± 0.9 | – | – |
| 20H | | | | | | | | | | | | |
| cy17:0 | 2.41 ± 1.2 | – | 2.15 ± 2.5 | 1.21 ± 0.8 | 0.73 ± 1.3 | 1.38 ± 1.5 | 4.76 ± 2.3 | – | 2.81 ± 1.9 | – | 3.03 ± 1.1 | – |
| cy19:0 | 4.59 ± 2.8 | – | 2.14 ± 1.4 | – | 3.54 ± 1.3 | – | 5.81 ± 2.6 | – | 3.53 ± 2.4 | – | 5.76 ± 2.6 | 1.86 ± 2.6 |
| 14:1ω7c | 1.05 ± 1.5 | 4.23 ± 0.6 | 0.82 ± 1.4 | 2.13 ± 0.9 | – | 2.85 ± 0.3 | 0.55 ± 0.9 | 3.38 ± 3.2 | 0.40 ± 0.8 | – | 2.20 ± 3.1 | 3.15 ± 0.1 |
| 16:1ω7c | – | – | 6.64 ± 1.1 | – | 3.27 ± 4.2 | – | 3.65 ± 1.1 | – | 0.58 ± 1.2 | – | – | – |
| 18:1ω9c | 1.42 ± 0.3 | – | 3.66 ± 0.5 | – | 2.94 ± 0.9 | – | 3.61 ± 2.1 | – | 0.94 ± 1.1 | – | 1.08 ± 1.5 | – |
| 18:2ω6c | 7.20 ± 3.9 | 5.93 ± 0.3 | 0.62 ± 1.1 | 5.19 ± 4.6 | 2.66 ± 3.5 | 5.62 ± 1.9 | 3.59 ± 4.1 | 3.33 ± 2.9 | 5.61 ± 4.5 | 1.18 ± 0.5 | 5.32 ± 3.6 | 1.42 ± 2.0 |
| 18:3ω6c | 0.91 ± 1.3 | – | 0.98 ± 1.7 | – | 0.70 ± 1.2 | – | 2.03 ± 0.5 | – | 0.87 ± 1.0 | 0.97 ± 1.7 | 1.31 ± 0.5 | 1.12 ± 1.6 |
| a15:0 | 2.04 ± 0.4 | 0.28 ± 0.8 | 4.43 ± 1.1 | 0.26 ± 0.5 | 4.29 ± 1.9 | 0.86 ± 1.5 | 3.39 ± 1.4 | 0.21 ± 0.2 | 2.58 ± 1.7 | 0.20 ± 0.6 | 3.63 ± 1.7 | 0.28 ± 0.5 |
| i15:0 | 4.49 ± 1.8 | 0.35 ± 0.2 | 5.64 ± 1.4 | 0.39 ± 0.2 | 5.57 ± 2.5 | 1.92 ± 1.8 | 5.98 ± 2.2 | 2.01 ± 0.3 | 3.63 ± 2.5 | 0.61 ± 0.2 | 6.12 ± 2.5 | 2.10 ± 0.6 |
| i16:0 | 1.12 ± 0.6 | – | 1.48 ± 1.3 | – | – | – | 2.55 ± 1.3 | – | 1.12 ± 0.8 | – | – | – |
| a17:0 | 0.86 ± 1.2 | 0.86 ± 0.9 | 0.84 ± 1.5 | 1.01 ± 0.6 | 0.47 ± 0.8 | – | 1.00 ± 0.9 | 0.28 ± 0.6 | 0.79 ± 0.9 | 0.43 ± 0.2 | – | 1.25 ± 0.5 |
| i17:0 | 1.35 ± 1.9 | 0.95 ± 0.5 | 1.85 ± 1.6 | 0.56 ± 0.2 | – | – | 1.88 ± 1.7 | 0.41 ± 0.2 | – | 0.72 ± 0.3 | – | 0.78 ± 0.2 |
| i19:0 | 1.11 ± 1.6 | – | – | 0.21 ± 0.6 | – | – | 0.81 ± 1.4 | – | 1.20 ± 1.6 | – | – | 0.55 ± 0.6 |
| 14:0 | 3.88 ± 0.5 | 6.17 ± 0.5 | 5.06 ± 0.8 | 3.79 ± 3.5 | 5.51 ± 1.9 | 6.26 ± 0.9 | 3.62 ± 0.9 | 4.06 ± 3.6 | 5.19 ± 2.2 | 0.37 ± 0.6 | 5.48 ± 1.2 | 3.86 ± 1.0 |
| 15:0 | – | 1.53 ± 2.7 | – | – | – | – | 0.85 ± 0.8 | – | 0.77 ± 0.9 | – | – | – |
| 16:0 | 14.9 ± 1.3 | 23.1 ± 0.4 | 19.6 ± 2.4 | 19.3 ± 2.7 | 19.4 ± 7.7 | 20.4 ± 2.6 | 16.7 ± 5.1 | 22.6 ± 6.3 | 18.7 ± 2.7 | 13.2 ± 9.8 | 21.2 ± 4.3 | 17.7 ± 0.1 |
| 18:0 | 5.70 ± 0.2 | 15.2 ± 2.6 | 7.78 ± 1.8 | 13.3 ± 1.6 | 9.04 ± 3.7 | 11.9 ± 1.7 | 5.50 ± 1.1 | 11.3 ± 2.7 | 6.48 ± 3.7 | 10.9 ± 9.8 | 8.38 ± 2.9 | 10.7 ± 1.3 |
| 20:0 | 0.88 ± 1.2 | – | – | – | – | – | 0.45 ± 0.8 | – | – | – | – | – |
| Total | 61.34 | 40.32 | 65.55 | 47.07 | 63.42 | 51.22 | 71.41 | 53.08 | 57.38 | 28.79 | 52.97 | 47.78 |

PLFA^c^aFatty acid contents under different plants and a control soil with the most significant contributions to the separation of Palouse soil from Ritzville soil.^bExpressed as % total PLFA ($n = 3$).

–, concentrations below detection limits by instrument.

^cTotal amount of PLFA in nmol g^{-1} dry wt.

Based on this information, fatty acids with the highest loading rate were used to construct the bi-plot. We chose fatty acids with correlation $r \geq 0.55$ or -0.55 . PCA explains the variance-covariance structure through a few linear combinations of the original variance (PLFA) with coefficients equal to the eigenvectors of the correlation matrix [20]. Total microbial biomass was determined by the method of White et al. [21].

The patterns of sole carbon source utilization in Biolog plates were analyzed using three approaches. The first two approaches used binary method and raw data to separate the samples. The third approach was the use of weighted-averages transformations of data prior to data analysis and expressed as an index of color development in each of the wells. The index was obtained by subtracting the color response reading from the spectrophotometer of each

of the 95 wells from the control well [14], then dividing by the control well according to the formula: $W_E = (W_A - W_0 / W_0) 100$. W_A = number of wells from A_2 to H_{12} and W_0 is the blank well. W_E greater than 100 was regarded as a positive reaction (evidence of substrate utilization) and W_E less than 100 was regarded as a negative reaction. The variables were coded by binary values (1 for positive and 0 for negative reaction). This approach was used to eliminate bias between samples with different numbers of culturable microorganisms and produced a weighted data set that can be used for analysis by PCA.

2.6. Fatty acid nomenclature

Fatty acids are designated by the number of carbon atoms, followed by a colon, the number of dou-

Table 2

Phospholipid fatty acid contents of soils under wheat plants and a control soil from field and greenhouse Ritzville soil^a

| PLFA ^b | Percent total LFA composition (mean \pm S.D.) ^c | | | |
|-------------------------|--|-----------------|------------------|-----------------|
| | Wheat | | Control | |
| | Field | Green house | Field | Green house |
| 16:0 2OH | – | 1.21 \pm 0.92 | – | – |
| cy17:0 | 3.69 \pm 2.15 | 5.41 \pm 2.31 | 1.03 \pm 0.31 | – |
| cy19:0 | 4.51 \pm 3.24 | 2.34 \pm 3.41 | 1.36 \pm 0.41 | 0.86 \pm 0.6 |
| 14:1 ω 7c | 3.54 \pm 2.31 | 2.58 \pm 0.36 | 2.20 \pm 3.1 | 2.12 \pm 2.10 |
| 16:1 ω 7c | 5.61 \pm 0.9 | 1.81 \pm 3.11 | 6.15 \pm 0.36 | 5.85 \pm 1.58 |
| 18:1 ω 9c | 1.25 \pm 0.25 | 4.36 \pm 2.14 | 2.37 \pm 0.84 | 1.20 \pm 0.69 |
| 18:2 ω 6c | 0.95 \pm 0.45 | 5.64 \pm 2.78 | 3.24 \pm 1.25 | 3.32 \pm 4.47 |
| 18:3 ω 6c | 1.02 \pm 0.30 | 1.09 \pm 0.89 | 0.26 \pm 0.05 | 0.65 \pm 0.12 |
| a15:0 | 1.28 \pm 0.89 | 2.13 \pm 1.94 | 1.23 \pm 0.39 | – |
| i15:0 | 0.59 \pm 0.05 | 2.72 \pm 2.60 | 2.05 \pm 1.23 | 1.23 \pm 2.13 |
| i16:0 | – | – | – | – |
| a17:0 | 1.21 \pm 0.05 | – | 0.24 \pm 0.25 | 0.98 \pm 1.05 |
| i17:0 | – | – | – | 0.89 \pm 0.26 |
| i19:0 | 0.12 \pm 0.32 | – | – | – |
| 14:0 | 3.89 \pm 2.14 | 3.39 \pm 2.22 | 2.58 \pm 1.54 | 3.99 \pm 5.64 |
| 15:0 | 0.88 \pm 0.10 | – | 0.77 \pm 0.56 | – |
| 16:0 | 10.56 \pm 5.27 | 8.29 \pm 3.56 | 12.87 \pm 4.35 | 8.78 \pm 3.25 |
| 18:0 | 1.81 \pm 2.12 | 0.98 \pm 0.8 | 0.58 \pm 1.02 | 0.95 \pm 1.02 |
| 20:0 | – | – | 0.98 \pm 1.45 | – |
| Total PLFA ^d | 47.38 | 46.85 | 40.44 | 33.47 |

^aSamples were collected from wheat field and control soil only.^bFatty acid contents under wheat plants and a control soil with the most significant contributions to the separation of Ritzville soil from Palouse.^cExpressed as % total PLFA ($n=3$).

–, concentrations below detection limits by instrument.

^dTotal amount of PLFA in nmol g⁻¹ dry wt.

ble bonds and then by the position of the first double bond from the methyl (ω) end of the molecules. *cis* and *trans* isomers are indicated by c or t. Branched chain fatty acids are indicated by the prefixes ‘i’ and ‘a’ for *iso* and *anteiso* branching, respectively. The prefix ‘cy’ designates cyclopropane fatty acid.

3. Results

3.1. PLFA composition

The percent composition and the numbers of different PLFAs from Palouse and Ritzville soils with the most significant contribution to the separation of field samples were on the average higher than those from greenhouse samples (Tables 1 and 2). Cyclopropane PLFAs (cy17:0 and cy19:0), characteristic of Gram-negative bacteria [22] were higher in the

field samples than the greenhouse samples. Samples obtained from barley, pea and wheat soils were higher in cyclopropane fatty acids than the control, while downy brome and jointed goatgrass showed lower percent composition in this group than the control soil. There were differences in monounsaturated fatty acids (14:1 ω 7c, 16:1 ω 7c, and 18:1 ω 9c) among downy brome, jointed goatgrass and pea soils from the greenhouse compared to the control soil. Straight chain PLFAs had the highest percent concentration in all the samples, with 16:0 having the highest percent concentration. Branched PLFAs (a15:0, i15:0, a17:0, and i17:0), characteristic of Gram-positive bacteria [23], were about three- to five-fold higher in soils under plant influence in the field than the control soil, and were all higher in percent concentrations than samples from the greenhouse soils. The polyunsaturated PLFAs, mostly 18:2 ω 6c and 18:3 ω 6c, characteristic of fungi [24], did not show

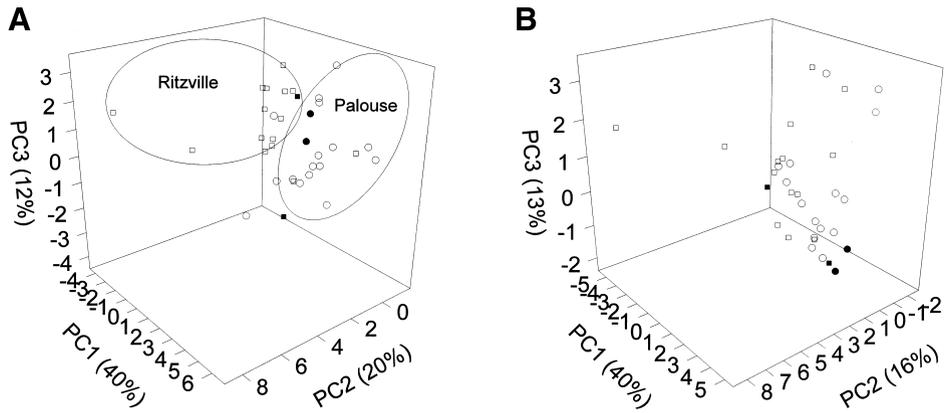


Fig. 1. A: Whole soil communities and B: plated communities of PLFA profiles of two soils from eastern Washington State. ○, Palouse soil under plant influence; ●, Palouse soil with no plant (control); □, Ritzville soil (covered with wheat); and ■, Ritzville soil (fallow plot). Eighteen fatty acids with the most significant contributions were used for these plots.

any pattern indicating they were not influenced by plant or by environment. In general, the percent concentration of hydroxy, cyclopropane, and branched PLFAs were highest in the field soils than either the control or the greenhouse soils, indicating differences in sources of different species contributing to these communities.

3.2. Biomass

PLFA content in different field and control soils as determined by microbial biomass per g dry weight showed marked variations, ranging from 28.79 nmol g⁻¹ dry wt in the greenhouse-Palouse soil with wheat to 71.41 nmol g⁻¹ dry wt in the field-Palouse soil with pea. Biomass was higher in all the field samples than greenhouse samples, and no differences were found in biomass between the two greenhouse soils. However, changes in biomass between Ritzville field and greenhouse soils were not as high as was found in the Palouse soil. The variation in biomass composition from the different environments was in agreement with the clusters obtained in Figs. 2A and 3A for field and greenhouse samples. The PLFA contents in soils under different plants in clusters labeled field-Palouse were higher than that from greenhouse-Palouse cluster. The same effects were seen in Ritzville soils. The decrease in microbial biomass in the greenhouse samples suggests a possible change in microbial composition when the same

soils were used for both greenhouse and field experiments.

3.3. Microbial community structure

Principal component analyses of whole soil and plated communities (Fig. 1A and B) were conducted to determine microbial community structure of Palouse and Ritzville soils. PCA was conducted with 29 PLFAs that were present in whole soil communities and with 37 PLFAs from plated communities. This explained 48% of the total variance in the first three dimensions for whole soil communities and 44% of the total variance in the plate communities. PCA of whole soil communities revealed separation of the two soils into two clusters (Fig. 1A), while no separation of the soils was obtained when plated communities were used. Due to the low amount of variance explained by the first three dimensions of the PCA, we ran PCA on matrices with fewer PLFAs. Eighteen PLFAs with the most significant contributions to the PCA ($r \geq 0.55$ or -0.55) were selected. However, the 3-D plot constructed from the reduced matrix did not show any new clusters (data not shown). Also, PLFAs with the most significant contributions to each of the principal component scores were the same for both the reduced data set and with all the PLFAs. Branched, monounsaturated, cyclopropane PLFAs were heavily weighted on PC1 = a15:0, i15:0, i16:0, 16:1 ω 7c, a17:0, i17:0,

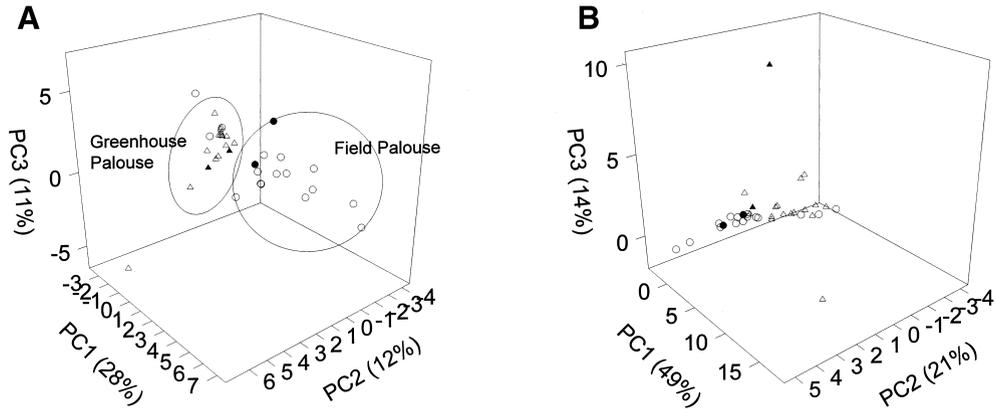


Fig. 2. A: Whole soil communities and B: plated communities showing PLFA profiles of samples obtained from Palouse soil in the field and in the greenhouse: ○, field samples with plants; ●, field samples without plants (control); △, greenhouse samples with plants; ▲, greenhouse samples without plants (control).

cy17:0, 18:1 ω 9c, and cy19:0. Most of the fatty acids in PC1 are of bacterial origin. PC2 and PC3 were heavily weighted by straight chain and one branched chain PLFA (PC2=12:0, a13:0, 15:0, 17:0, 20:0; PC3=14:0 and 16:0; Table 3). It should be noted that most of the PLFAs were dominated by straight chain fatty acids (40%) in the whole soil communities, and by branched chain fatty acids (50%) in the plated communities with a15:0 and i15:0 constituting the highest percent concentrations (data not shown).

To examine microbial community structure and

composition between field samples and greenhouse samples, PCA was constructed with field and greenhouse samples from Palouse and Ritzville soils. PCA plot of PLFA profiles from these soils showed two clusters. The field samples from Palouse and Ritzville soils were on the right of the PCA plot while greenhouse samples were on the left (Figs. 2A and 3A). When plated communities were plotted there was no separation of field and greenhouse soils (Figs. 2B and 3B). This indicates that culturable microorganisms may not give a good indication of community structure, since a small percent of the total popula-

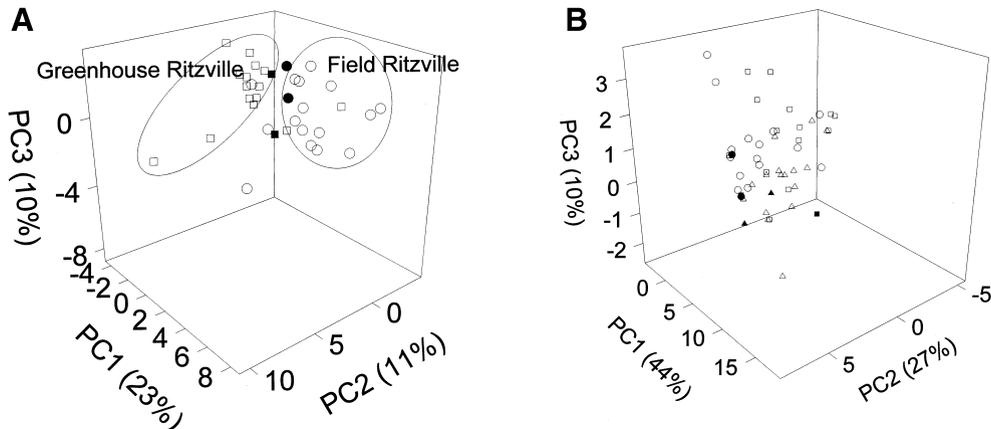


Fig. 3. A: Whole soil community PLFA profile and B: plated community PLFA profiles of samples obtained from Ritzville soil in the field and greenhouse. ○, field samples with plants; ●, field samples without plants; □, greenhouse samples with plants; ■, greenhouse samples without plants (control).

tion is culturable. The clusters obtained from the soil communities indicate differences in the species compositions, as well as differences in microbial biomass in the two soils.

To find the relative proportions of individual PLFAs contributing most to the differentiation of the two soil environments, we computed the correlation between principal components and PLFAs for PC1, PC2, and PC3. Nineteen PLFAs with correlation $r \geq 0.55$ or -0.55 were selected with their percent total PLFA concentrations (Table 3). The relative compositions of these PLFAs (PC1) showed that samples from the field had higher percent PLFA composition than those from the greenhouse environments, indicating higher numbers of microbial cells and biomass in the field than the greenhouse. This is in agreement with the clusters in Figs. 2A and 3A which clearly showed that the two environments are different. Most of the PLFAs in PC1 are of bac-

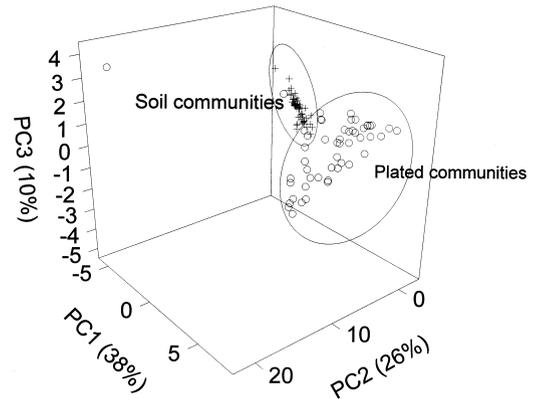


Fig. 4. PLFA profiles from (+) whole soil communities and (O) plated communities from all the samples used in this study.

terial origin except 18:2 ω 6c (fungi) and 18:0 (general). In general, these bacteria biomarkers in PC1

Table 3

Phospholipid fatty acids with the most significant contributions to PCA plot (Fig. 2a) from field and greenhouse (GH) soils combined from five plants^a

| PLFA | <i>r</i> | Percent total PLFA | | | |
|------------------|----------|--------------------|--------------|-----------------|--------------|
| | | Field-Palouse | GH-Palouse | Field-Ritzville | GH-Ritzville |
| PC1 | | | | | |
| a15:0 | 0.75 | 3.38 ± 0.86 | 0.98 ± 1.50 | 4.21 ± 1.26 | 1.73 ± 1.38 |
| i15:0 | 0.73 | 5.23 ± 0.28 | 1.12 ± 0.85 | 4.61 ± 1.91 | 2.16 ± 1.78 |
| 16:0 2OH | 0.61 | 1.44 ± 0.56 | 2.28 ± 0.20 | 1.12 ± 0.12 | 0.09 ± 0.16 |
| i16:0i | 0.80 | 1.25 ± 0.74 | – | 0.92 ± 0.21 | 0.19 ± 0.12 |
| 16:1 ω 7c | 0.71 | 2.82 ± 1.48 | – | 1.78 ± 0.81 | 0.45 ± 0.77 |
| a17:0 | 0.65 | 0.79 ± 1.06 | – | 1.21 ± 0.28 | 0.10 ± 0.12 |
| cy17:0 | 0.87 | 2.57 ± 1.78 | 0.52 ± 0.21 | 1.71 ± 0.71 | 2.00 ± 0.40 |
| i17:0 | 0.70 | 2.02 ± 1.10 | – | 1.51 ± 0.52 | – |
| 18:0 | –0.57 | 6.90 ± 2.10 | 12.10 ± 1.21 | 15.15 ± 3.82 | 17.69 ± 4.29 |
| 18:1 ω 9c | 0.83 | 2.51 ± 0.97 | – | 2.15 ± 0.37 | 0.48 ± 0.84 |
| 18:2 ω 6c | 0.58 | 3.94 ± 3.41 | 4.01 ± 1.93 | 6.21 ± 2.80 | 6.23 ± 3.80 |
| cy19:0 | 0.71 | 3.92 ± 2.25 | – | 2.37 ± 1.51 | – |
| PC2 | | | | | |
| 12:0 | 0.77 | 0.11 ± 0.18 | – | 0.67 ± 0.21 | 0.10 ± 0.11 |
| a13:0 | 0.78 | – | – | 1.21 ± 0.62 | 0.57 ± 0.14 |
| 17:0 | 0.72 | – | – | 0.83 ± 0.52 | 0.36 ± 0.62 |
| 20:0 | 0.76 | 0.27 ± 0.41 | – | 0.38 ± 0.11 | 0.31 ± 0.29 |
| PC3 | | | | | |
| 14:0 | 0.64 | 14.65 ± 1.25 | 4.13 ± 1.82 | 6.21 ± 2.10 | 4.75 ± 2.78 |
| 16:0 | 0.58 | 17.86 ± 3.84 | 19.71 ± 6.37 | 18.75 ± 4.63 | 17.86 ± 3.84 |
| 19:1 ω 11 | –0.64 | – | 0.22 ± 0.38 | 0.21 ± 0.09 | – |

^aData from Ritzville field soil were collected only under wheat plants.

–, concentrations below detection limit by instrument.

were higher in the field than in the greenhouse samples. PC2 and PC3 used mainly biomarkers of general microeukaryote origin.

To examine what portion of the community was culturable, PCA was conducted with plated organisms and the whole soil community. The PCA plot of the plated organisms was separated from that of the whole soil community (Fig. 4). Only a fraction of the plated community shared the same cluster with the soil community. This suggests that PLFA profiles from soil communities were different from the plated community and that only a portion of the soil communities was culturable.

The second approach for community analysis in this study was the use of patterns of potential C source utilization. Our primary goal was to evaluate the effectiveness of three statistical approaches: (a) use of binary numbers, 0 for a negative reading (no substrate utilized) and 1 for a positive reading (substrate utilized) when plates were observed; (b) raw differences, by subtracting the readings from each of the substrate wells from the control well and using that data for analysis; (c) weighted-averages transformation of Biolog data after 72 h of incubation. Analysis of patterns of C utilization using the weighted-averages transformation revealed consistent soil differences based on environment (Fig. 5).

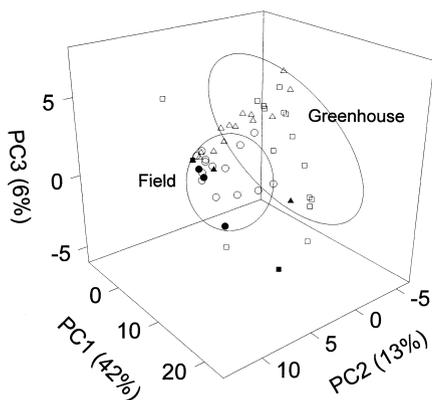


Fig. 5. C source utilization patterns from Palouse and Ritzville soils: Greenhouse Palouse and Ritzville soils under plants (○) and Palouse and Ritzville soils without plants (●). Field: △, Ritzville soil under plants; and ▼, Ritzville soils without plants; □, Palouse soil under plants; and ■, Palouse soil without plants after 72 h incubation at 25°C. Greenhouse data were pooled since there was no separation of data points.

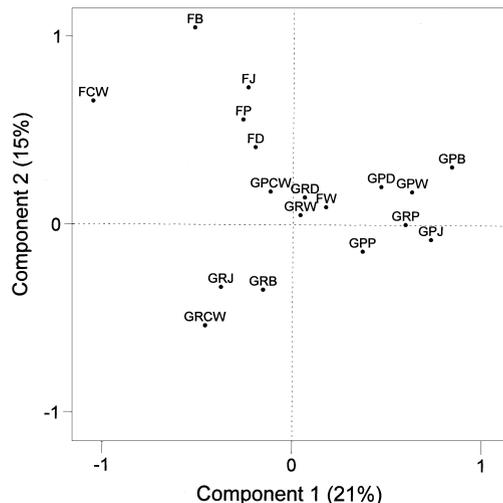


Fig. 6. Results from PCA of field rhizosphere samples from barley (FB), jointed goatgrass (FJ), pea (FP), downy brome (FD), wheat (FW) and greenhouse rhizospheres samples from Palouse and Ritzville soils. Last letters of abbreviation signify same plants as above, GP and GR for greenhouse Palouse and Ritzville respectively. C indicates control soil.

The field samples from Palouse and Ritzville soils were mainly in one cluster while the greenhouse samples from Palouse and Ritzville soils were in another cluster. This was in agreement with our PLFA data. Data from the first two approaches did not distinguish the two soils (data not shown).

The 72 h time point was chosen for our analysis because at this time 75% or more of our wells had produced a positive reaction to the substrates which may represent a condition where most of the microorganisms had responded to the substrate. When PCA was conducted with a 48 h time point, the data points did not separate which suggests that a longer time point must be used when analyzing complex environments. Recently, Knight et al. [25] used a time point of 60 h of incubation, and suggested that greatest differences in the response of microbial extracts to substrates were observed at this point.

The separation of field and greenhouse samples (Fig. 5) revealed that most of the field samples clustered in one section of the plot. A second PCA was conducted to determine the patterns of clustering of individual plants and their source of nutrients based on the substrate utilization patterns of the soils of these plants. In the PCA score plot (Fig. 6), which

included the samples from all plants from field and greenhouse, the effects of the greenhouse environment on Palouse soil and half of the Ritzville soil were positively correlated to the right of PC1 and the other half of the Ritzville soil negatively correlated to the left of PC1. The effect of field environment was positively correlated on PC2. Most of the field samples were found in vector one of the PC2 (positive side of PC2). This is also in agreement with our PLFA data which showed all field samples in one cluster. Analysis of each cluster indicated that 20 substrates were used in field samples (vector 1, Fig. 6) with correlation (r) greater than 0.50. This information was revealed by coding each substrate positive (1) or negative (0) once data were standardized.

4. Discussion

PLFA and carbon source utilization were used to describe microbial community structure of the two soils. The use of PLFA profiles is based on the fact that phospholipids are found in the membrane of living cells, and bacteria contain a relatively constant proportion of their biomass as phospholipids. Since some bacteria have unique signature PLFA profiles which can be used to examine community structure [26,27], we used PLFA profiles from whole soil and plated communities to examine soil microbial community structure. Changes in PLFA composition and biomass observed in this study provide some evidence of changes in microbial community structure and composition related to plant growth environments (field and greenhouse). Our results suggest that greenhouse soils support lower microbial biomass than field soils. For example total PLFA contents, which reflect active microbial biomass [28–30], declined from 71.41 nmol per g dry wt in pea soil in the field to 53.08 nmol per g dry wt in pea soil in the greenhouse. This trend was observed in all the greenhouse samples compared to the field samples (Tables 1 and 2).

The interpretation of the changes in patterns of PLFA in soils in terms of changes in specific taxonomic groups is difficult since the same PLFA marker may exist in the membranes of organisms belonging to different taxonomic groups. However, certain trends can be pointed out in terms of certain

PLFAs belonging to certain groups of bacteria. It is well established that certain branched, monounsaturated, and cyclopropane fatty acids present in environmental samples are contributed by the in situ bacterial population. Branched fatty acids (a15:0, i15:0, a17:0, and i17:0) had higher percent total PLFA in field samples than greenhouse samples. Branched fatty acids have been reported as biomarkers for bacteria [31], anaerobic bacteria [32,33], and sulfate-reducing bacteria *Desulfo vibro* spp [34].

The microbial community structure presented in Fig. 2A, Fig. 3A and Table 3 illustrates how shifts in microbial community structure affect PLFA composition (Table 3). The separation by PCA (Figs. 2A and 3A) suggests that there are differences in community composition between the two environments. This was further illustrated by the percent of individual PLFAs contributing to each of the three PC scores. PC1 was heavily weighted by fatty acids that are of bacterial origin except 18:2 ω 6c. The percent of these fatty acids was higher in the field samples than greenhouse samples. The inability of PCA to separate cultured bacterial communities suggests that the culturable fraction from plated communities may represent closely associated microorganisms from the two environments. When whole soil and plated communities were plotted together (Fig. 4), there was a separation of the two communities, suggesting that the whole soil community is different from the plated community. One possible explanation for this is that whole soil community profiles captured larger portions of soil microbial communities that were not culturable. This was in agreement with Cavigelli et al. [35], who showed differences in FAME profiles between soil and plated communities when microbial communities from the same samples were cultured and FAME profiles analyzed. This is probably the main reason we could not use plated communities to describe soil microbial community structure from different soils and environments.

The quantification of environmental samples analyzed on the basis of carbon source utilization in Biolog plates provided a powerful tool for describing soil microbial communities. The analysis of both the field and the greenhouse samples using the PLFA and the Biolog community assay for soil microbial community analysis provides more information to

answer some of the questions raised in the literature. One of those questions is the problem of inoculation density raised by Haack et al. [15]. The net effect is that the Biolog system reflects the metabolic activity of a certain proportion of the community. The use of weighted-averages transformed data with the variables subsequently coded by binary values (1 for values greater than 100 and 0 for values lesser than 100) was to uncover structural differences associated with large proportions of microorganisms associated with the soil environment. This was the only technique for data analysis that produced results similar to PLFA data.

Analysis of PC1 (Fig. 5) showed that soil microbial communities utilized 85 of the substrates with positive correlation $r \geq 0.50$. These carbon compounds may be regarded as the most important carbon sources that differentiated among communities. However, poor correlation did not mean poor utilization by samples, but rather their utilization rate was not used to differentiate the communities. Different substrates were utilized by microbes in soils under different plants from the two environments, but there were no clear groupings to indicate that a particular environment was favored by certain groups of substrates (data not shown).

The 72 h time point for the development of substrate utilization patterns for the two communities was used because of the time required for microbial growth in the soils to have substantial effects on color development and cell size. This is in agreement with the work of Wünsche et al. [14], who used two Gram-negative bacteria with completely different substrate utilization patterns, and showed that the influence of inoculation density was largely removed by extending the inoculation time over 48 h. These authors concluded that inoculation-dependent differences among the Biolog patterns were more or less leveled out after an extended incubation period, and the Biolog patterns most probably reflect the metabolic activities of the quantitatively dominating components of the microbial communities. Therefore, by using an extended incubation period and data transformation, we believe that some of the problems associated with inoculation density in describing soil microbial community structure with substrate utilization data is further clarified. This is supported by our finding that Biolog and PLFA data revealed similar

clusters. Although the two methods revealed the same pattern of changes due to environment, variation in the data was much smaller for the PLFA measurement than the Biolog measurement. This suggests that PLFA technique may be more sensitive in detecting changes in microbial community than the Biolog technique. One possible reason for this is that PLFA measures the entire community while Biolog only measures a fraction of the community measured by PLFA.

The use of PLFA and C source utilization patterns suggests that these are useful techniques to describe soil microbial community structure once data are analyzed correctly and the proper incubation time used. These two methods were effective in distinguishing between the effects of different environment and the origin of samples on soil microbial community structure. Therefore, with the appropriate method of data analysis, the rapid nature of Biolog assay can provide useful information in support of other assays such as PLFA and rDNA analysis for microbial community structure characterization.

References

- [1] Alexander, M. (1977) *Introduction to Soil Microbiology*. John Wiley and Sons, New York.
- [2] Findlay, R.H. (1996) The use of phospholipid fatty acids to determine microbial community structure. In: *Molecular Microbial Ecology Manual* (Akkermans, A.D.L., Van Elsas, J.D. and de Bruijn, F, Eds.), pp. 4.1.4/1–4.1.4/17. Kluwer Academic Publishers, Dordrecht.
- [3] Amann, R.L., Ludwig, W. and Schleifer, K.H. (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* 59, 143–169.
- [4] White, D.C. and Findlay, R.H. (1988) Biochemical markers for measurement of predation effects on the biomass, community structure, nutritional status, and metabolic activity of microbial biofilms. *Hydrobiologia* 159, 119–132.
- [5] Balkwill, D.L., Leach, F.L., Wilson, T.J., McNabb, J.F. and White, D.C. (1988) Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate, and direct counts in the subsurface aquifer sediments. *Microbiol. Ecol.* 16, 73–84.
- [6] Rajendran, N., Matsuda, O., Imamura, N. and Urushigawa, Y. (1992) Variation in microbial biomass and community in the sediments of eutrophic bays as described by phospholipid ester-linked fatty acids. *Appl. Environ. Microbiol.* 58, 562–571.
- [7] Ringelberg, D.B., Davis, J.D., Smith, G.A., Pffner, S.M., Nichols, P.D., Nickels, J.S., Henson, J.M., Wilson, J.T.,

- Yates, M., Kampbell, D.H., Reed, H.W., Stocksdale, T.T. and White, D.C. (1988) Validation of signature phospholipid fatty acids biomarkers for alkaline-utilizing bacteria in soil and subsurface aquifer materials. *FEMS Microbiol. Ecol.* 62, 39–50.
- [8] 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.
- [9] Zelles, L., Rackwitz, R., Bai, Q.Y., Beck, T. and Beese, F. (1995) Discrimination of microbial diversity by fatty acid profiles of phospholipids and lipopolysaccharides in differently cultivated soils. *Plant Soil.* 170, 115–122.
- [10] Garland, J.L. and Mills, A.L. (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level-sole-carbon-source-utilization. *Appl. Environ. Microbiol.* 57, 2351–2359.
- [11] Garland, J.L. and Mills, A.L. (1994) A community-level physiological approach for studying microbial communities. In: *Beyond the Biomass: Composition and Functional Analysis of Soil Microbiol Communities* (Ritz, K., Dighton, J. and Giller, K.E., Eds.), pp. 77–83. John Wiley and Sons, Chichester, UK.
- [12] Garland, J.L. (1996) Patterns of potential C source utilization by rhizosphere communities. *Soil Biol. Biochem.* 26, 223–230.
- [13] Zak, J.C., Willing, M.R., Moorehead, D.L. and Wildman, H.G. (1994) Functional diversity of microbial communities: a quantitative approach. *Soil Biol. Biochem.* 26, 1101–1108.
- [14] Wünsche, L., Brüggermann, L. and Wolfgang, B. (1995) Determination of substrate utilization patterns of soil microbial communities: An approach to assess population changes after hydrocarbon pollution. *FEMS Microbiol. Ecol.* 17, 295–306.
- [15] Haack, S.K., Garchow, H., Odelson, D.L., Forney, L.J. and Klug, M.J. (1995) Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. *Appl. Environ. Microbiol.* 61, 1458–1468.
- [16] 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.
- [17] Smith, G.A., Nichols, P.D. and White, D.C. (1986) Fatty acid composition and microbial activity of benthic marine sediment from McMurdo Sound, Antarctica. *FEMS Microbiol. Ecol.* 38, 219–231.
- [18] Sherlock Microbial Identification System (1996) Operating Manual, version 6. Newark, Delaware.
- [19] SAS Institute (1988) Users guide: statistic version 6. Statistical Analytical Inst., Carey, NC.
- [20] Jolliffe, I.T. (1986) Principal Component Analysis. Springer-Verlag, New York.
- [21] White, D.C., Davis, W.M., Nickels, J.S., King, J.D. and Bobrie, R.J. (1979) Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia* 40, 51–62.
- [22] Wilkinson, S.G. (1988) Gram-negative bacteria. In: *Microbial Lipids* (Ratledge, C. and Wilkinson, S.G., Eds.), Vol. 1, pp. 299–488. Academic Press, London.
- [23] Ratledge, C. and Wilkinson, S.G. (1988) *Microbial Lipids*, Vol. 1. Academic Press, New York.
- [24] Federle, T.W. (1986) Microbial distribution in soil—new techniques. In: *Perspective in Microbial Ecology* (Megusar, F. and Gantar, M., Eds.), pp. 493–498. Slovene Society for Microbiology, Ljubljana.
- [25] Knight, B.P., McGrath, S.P. and Chaudri, A.M. (1997) Biomass carbon measurements and substrate utilization patterns of microbial populations from soils amended with cadmium, copper, and zinc. *Appl. Environ. Microbiol.* 63, 39–43.
- [26] Bååth, E., Frostegård, Å. and Fritze, H. (1992) Soil bacterial biomass, activity, phospholipid fatty acid pattern, and pH tolerance in an area polluted with alkaline dust deposit. *Appl. Environ. Microbiol.* 58, 4026–4031.
- [27] Tunlid, A., Hoitink, A.J., Low, C. and White, D.C. (1989) Characterization of bacteria that suppress *Rhizoctonia* damping-off in bark compost media by analysis of fatty acid biomarkers. *Appl. Environ. Microbiol.* 55, 1368–1374.
- [28] 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.
- [29] Tunlid, A. and White, D.C. (1992) Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial community in soil. In: *Soil Biochemistry* (Stotzky, G. and Bollag, J.-M., Eds.), Vol. 7, pp. 229–262. Marcel Dekker, New York.
- [30] Vestal, J.R. and White, D.C. (1989) Lipid analysis in microbial ecology. *Bioscience* 39, 535–541.
- [31] White, D.C. (1983) Analysis of microorganisms in terms of quantity and activity in natural environments. *Soc. Gen. Microbiol. Symp.* 34, 37–66.
- [32] Guckert, J.B., Hood, M.A. and White, D.C. (1986) Phospholipid ester-linked fatty acid profiles changes during nutrient deprivation of *Vibrio cholerae*: increases in the *trans/cis* ratio and proportions of cyclopropyl fatty acids. *Appl. Environ. Microbiol.* 52, 794–801.
- [33] Guckert, J.B., Ringelberg, D.B., White, D.C., Hanson, R.S. and Bratina, B.J. (1991) Membrane fatty acids as phenotypic markers in the polyphasic taxonomy of methylotrophs within the Proteobacteria. *J. Gen. Microbiol.* 137, 2631–2641.
- [34] Boon, J.J., De Leeuw, J.W., van der Hoek, G.J. and Vosjan, J.H. (1977) Significance and taxonomic value of iso and anteiso monoenoic fatty acids and branched β -hydroxy acids in *Desulfovibrio desulfuricans*. *J. Bacteriol.* 129, 1183–1191.
- [35] Cavigelli, A.C., Robertson, G.P. and Klug, M.J. (1995) Fatty acid methyl ester (FAME) profiles as measure of soil microbial community structure. *Plant Soil.* 170, 99–113.