

Comparison of phospholipid fatty acid (PLFA) and total soil fatty acid methyl esters (TSFAME) for characterizing soil microbial communities

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Abstract

Phospholipid fatty acid (PLFA) and total soil fatty acid methyl esters (TSFAME), both lipid-based approaches used to characterize microbial communities, were compared with respect to their reliable detection limits, extraction precision, and ability to differentiate agricultural soils. Two sets of soil samples, representing seven crop types from California's Central Valley, were extracted using PLFA and TSFAME procedures. PLFA analysis required 10 times more soil than TSFAME analysis to obtain a reliable microbial community fingerprint and total fatty acid content measurement. Although less soil initially was extracted with TSFAME, total fatty acid (FA) content g^{-1} soil (DW) was more than 7-fold higher in TSFAME- versus PLFA-extracted samples. Sample extraction precision was much lower with TSFAME analysis than PLFA analysis, with the coefficient of variation between replicates being as much as 4-fold higher with TSFAME extraction. There were significant differences between PLFA- and TSFAME-extracted samples when biomarker pool sizes (mol% values) for bacteria, actinomycetes, and fungi were compared. Correspondence analysis (CA) of PLFA and TSFAME samples indicated that extraction method had the greatest influence on sample FA composition. Soil type also influenced FA composition, with samples grouping by soil type with both extraction methods. However, separate CAs of PLFA- and TSFAME extracted samples depicted strong differences in underlying sample groupings. Recommendations for the selection of extraction method are presented and discussed.

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1. Introduction

Recent methodological advances in soil microbial ecology are increasing our understanding of soil microbial community composition and how community composition relates to soil processes. Many of the new methods extract the cellular constituents of microorganisms directly from soil, eliminating the bias inherent in culture-based methods (Fry, 1982; Pedros-Alio, 1993). Due to their chemical diversity and cellular abundance, lipids and nucleic acids are particularly promising constituents for investigating and characterizing microbial communities.

Lipids, a major cellular component, constitute a wide variety of structurally and functionally diverse compounds. Using multivariate statistical analyses, this variation in fatty acid (FA) composition between microorganisms can be exploited, revealing differences between microbial communities (Macalady et al., 2000). In addition, some FAs are considered biomarkers for specific groups of microorganisms, based on their lipid profiles from pure culture (Zelles, 1999).

Phospholipid fatty acids (PLFA) are major cell membrane constituents, and their polar head groups and ester-linked side chains (i.e. FAs) vary in composition between eukaryotes and prokaryotes, as well as among many prokaryotic groups. These compounds rapidly degrade upon cell death (Pinkart et al., 2002), making them good indicators of living organisms (White et al., 1979). Phospholipids are extracted directly from soil, and following hydrolysis, their

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Table 1
Descriptions of soil samples used for detection limit, biomarker analysis, and sample precision studies

Sample name	Crop	Soil texture	Sampling location	Notes
Vineyard 1	Grapes	Sandy loam	8.0 km S of Fresno, CA	Conventional treatment
Vineyard 2	Grapes	Sandy loam	22.5 km SW of Fresno, CA	As with Vineyard 1, but field originally was ripped to hardpan (to 1.5 m) prior to planting
Safflower conv	Safflower	Silty loam	SAFS, UC Davis, CA	Conventional treatment
Safflower org	Safflower	Silty loam	SAFS, UC Davis, CA	Organic treatment
Fallow org	Fallow	Silty loam	SAFS, UC Davis, CA	Organic treatment

Samples were collected in the fall after at least one growing season with these crops.

FAs are released. The variation in FA composition provides a ‘fingerprint’ of the living microbial community and has been used to study soil microbial community changes in agricultural soils (Bossio et al., 1998; Calderon et al., 2001; Peacock et al., 2001) and heavy metal-contaminated soils (Bååth et al., 1995; Pennanen, 2001), amongst others.

In contrast to PLFA, with total soil fatty acid methyl ester (TSFAME) analysis all soil lipids are extracted and subsequently methylated to release their respective fatty acid methyl esters (FAMES). TSFAME extracts lipids derived from cellular storage compounds and cellular membranes, as well as components from living and dead microbial and animal cells and plant tissues in various stages of decomposition. Due to the complex nature of these extracts, it is more difficult to draw conclusions about changes in the extant microbial community based upon these profiles. However, extracting TSFAMES is more rapid than PLFA analysis and has been used to describe microbial communities in agricultural soils (Klug and Tiedje, 1993; Cavigelli et al., 1995; Buyer and Drinkwater, 1997; Ibekwe and Kennedy, 1999) and previously mined soils (Mummey et al., 2002).

Although laboratory-based studies comparing PLFA and FAME have been conducted on isolates (Haack et al., 1994), few studies have compared these two methods using the same soil samples or evaluated the relative strengths and weaknesses of the two methods for environmental sample analysis (Drijber et al., 2000; Pankhurst et al., 2001). Our objectives were to compare the TSFAME and PLFA methods with respect to their (1) reliable detection limits and extraction precision, (2) biomarker composition, and (3) ability to discriminate environmental samples. To address these objectives we determined method-specific detection limits based upon the amount of soil extracted and then used correspondence analysis to compare the abilities of these two methods to separate soil microbial communities based on soil and crop type.

2. Materials and methods

2.1. Soils and sampling

Two groups of soils were used in this study. The first group included five soils that varied by crop type, soil

texture and geographical region. These five soils were used to determine detection limits, sample-to-sample variability, and sample biomarker composition when soils were extracted by both methods. Three of the soils were from the University of California, Davis Sustainable Agriculture Farming Systems (SAFS) project (Scow et al., 1994; Temple et al., 1994; Gunapala and Scow, 1998) in California’s northern Central Valley, and two were from agricultural areas surrounding Fresno, in California’s southern Central Valley (Table 1). All samples were collected from the top 10–15 cm of soil. To decrease heterogeneity, soil samples were well-homogenized and sieved (2 mm) before air drying. Samples then were kept frozen at -20°C until extracted.

The second soil set contained a larger and more variable set of soils in order to determine the ability of the two methods to discriminate between environmental samples. This group was comprised of 55 soils obtained from the UC Davis Air Quality Group’s PM_{10} project (Johnson et al., 2003), consisting of air dried soils from agricultural fields in the San Joaquin Valley, CA (Table 2). These samples represented major crops within the valley (cotton, almond, walnut and fig) and were collected from field surfaces (top 10–15 cm), air dried, sieved (2 mm), and stored at ambient temperature until analysis.

Table 2
Descriptions of soil samples used for environmental sample discrimination

Crop	Soil texture	Number of sites	Number of samples per site
Almond	Clay loam	1	7
Almond	Loam	2	4
Almond	Sandy loam	2	5
Cotton	Clay	5	8
Cotton	Clay loam	1	2
Cotton	Loam	2	3
Cotton	Sandy loam	1	2
Cotton	Silty loam	1	3
Walnut	Loam	2	7
Walnut	Loamy sand	1	1
Walnut	Sandy loam	2	2
Fig	Loamy sand	2	2
Fig	Sandy loam	1	9

Samples were collected in the fall after at least one growing season with these crops.

2.2. Detection limit analysis

Different masses of each of the five soils were extracted and analyzed by both PLFA and TSFAME. These masses were determined from preliminary analyses and ranged from amounts yielding lipid concentrations near the GC detection limit to concentrations in excess of that required to reduce sample-to-sample variability. For PLFA extraction, the range of soil dry masses was from 0.1 to 8.0 g; for TSFAME, from 10 to 500 mg. Soil samples were extracted in duplicate for both methods, and the replicates were combined before statistical analysis.

2.3. PLFA extraction

Duplicate (detection limit analysis) or triplicate (sample discrimination analysis) subsamples of 8 g of soil (DW) (unless stated otherwise) were extracted with a one-phase solvent extractant, using a modification of the Bligh and Dyer (1959) method as described in Bossio and Scow (1995). Where lower soil masses were extracted (detection limit study), only the initial phosphate buffer volume was altered to account for soil moisture content. In subsequent sample preparation steps, solvent volumes were not modified based on initial soil mass extracted. Polar lipids were separated from neutral and glycolipids using solid-phase extraction columns (0.5 g of Si; Supelco Inc., Bellefonte, Pa.) by elution with 5 ml of chloroform, followed by 10 ml of acetone. Polar lipids (including phospholipids) were then eluted with 5 ml of methanol, and dried under N₂ at 37 °C. The resulting polar lipid fraction was then subjected to mild alkaline methanolysis, and the resulting fatty acid methyl esters (FAMES) were extracted with two 2 ml aliquots of hexane. Combined hexane aliquots were dried under N₂ at room temperature, and re-dissolved in hexane containing an internal standard (19:0 FAME at 25 ng ml⁻¹).

2.4. TSFAME extraction

Duplicate (detection limit analysis) or triplicate (sample discrimination analysis) subsamples of 0.5 g of soil (DW) (unless stated otherwise) were extracted according to the Microbial Identification System (MIS; Microbial ID Inc., Newark, DE) standard procedure. To each soil sample, 3.25 M NaOH in MeOH:H₂O (1:1) was added (1 ml solution added per 1 g soil). The samples were vortexed and then placed in an 80 °C water bath for 30 min, during which time the cells were lysed and the FAs were cleaved from the cellular lipids. Following this saponification step, the FAs were converted to FAMES by adding 6.0 M HCl:MeOH (1:0.85) (2 ml solution per 1 g soil) to each sample. To extract the FAMES from the acidic, aqueous phase into the organic phase a hexane:MTBE (1:1) solution was added to each sample (2 ml solution per 1 g soil). Following addition of the hexane:MTBE (1:1) solution, the MIDI procedure

then was modified as described in Cavigelli et al. (1995) by adding a 10 min 2500 rpm centrifugation step following extraction. The organic phase subsequently was removed, washed with a mild base (0.3 M NaOH), and dried with N₂ before re-dissolution in hexane containing an internal standard (19:0 FAME at 5 ng ml⁻¹).

2.5. Gas chromatography

All samples were analysed using a Hewlett Packard 6890 gas chromatograph with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column (J&W Scientific, Folsom, CA). A single 2 µl injection, with a 1:50 split, was analysed at an initial temperature of 170 °C and then ramped to 260 °C at 2 °C min⁻¹, at a constant flow rate of 5 ml min⁻¹. Peaks were identified using bacterial FA standards and MIDI peak identification software (MIS; Microbial ID Inc.).

2.6. Statistical analysis

Concentrations of each FA (mean nmol g⁻¹ soil (DW)) were calculated based on the 19:0 internal standard concentration. These concentrations were used in all statistical analyses. Analysis of variance (ANOVA) and correspondence analysis (CA) were performed using SAS (Version 8.0, SAS Institute, Cary, NC). Correspondence analysis was used to generate unconstrained ordination plots based on FA data, allowing interpretation of sample-to-sample relationships based on microbial community composition.

To assess or remove the effects of low yield FAs between samples, we compared two subsets of FAs in our analyses. The first subset included the FAs present in all soil samples analyzed (FA cutoff = 100%), and the second subset included the FAs present in at least half the samples analyzed (FA cutoff = 50%). These subsets were determined after averaging replicates.

Univariate ANOVA was used to compare the influence of extraction method on the relative pool sizes of biomarkers (i.e. mole percent values, mol mol⁻¹) within a soil type. The biomarkers analyzed within this dataset included 10Me FAs (indicative of actinomycetes), 18:2ω6,9c (indicative of fungi), and a summation of i15:0, a15:0, 15:0, i16:0, 16:1ω5c, i17:0, a17:0, 17:0cy, 18:1ω7c, 19:0cy (indicative of bacteria). It was observed that more short chain FAs (<14 C) were extracted with TSFAME than PLFA. Therefore, short chain FAs also were analyzed by ANOVA. As is often encountered with FA data, the dataset strongly violated the assumption of equal variance between treatments. Transformation failed to improve this situation. Therefore, soil types were analyzed separately. With only one effect in the model, weighted ANOVA could be used in cases in which there was still unequal variance between extraction method within a soil type.

3. Results

3.1. Detection limit analysis

Overall, both methods extracted similar numbers of FAs. Averaging results from the two highest sample masses extracted by each method, the mean number of FAs detected and identified was 34.3 for PLFA and 34.1 for TSFAME. Twenty-nine FAs were common to both methods when including all identified FAs. When including only FAs present in all of the samples, 23 FAs were common between both methods. However, total FA content was over 7-fold higher with TSFAME (219 nmol FA g⁻¹ DW) than PLFA (31 nmol FA g⁻¹ DW) (Tables 3 and 4).

Individual CAs evaluating TSFAME and PLFA extractions were performed for each soil type. Comparing all soil masses extracted by both methods allowed us to determine the minimum sample mass required to retain a reliable community profile. Overall, the patterns obtained were similar between soil samples, and therefore, only one plot (Vineyard 1) is presented (Fig. 1, cutoff = 50%). Nearly all of the variation in the sample-to-sample relationships (based on their FA composition) was explained by the first two axes in this analysis (Fig. 1). However, the results were not as expected; rather than a gradual shift in FA composition with decreasing sample mass (and thus a gradual shift in sample pattern), high mass samples and low mass samples grouped separately. All low mass samples grouped together, regardless of extraction method. However, the high mass samples grouped based on extraction method, revealing differences in their underlying FA composition. Across all soil types, on average ≥ 1.1 g DW soil for PLFA and ≥ 140 mg DW soil for TSFAME was required to maintain a reliable microbial community fingerprint (data not shown); extracting lower soil masses missed many low-yielding FAs and therefore provided an incomplete FA fingerprint.

Table 3

Detection limit analysis: minimum sample mass required to obtain mean total FA contents (nmol FA g⁻¹ DW soil) similar to those measured when recommended soil masses were extracted by PLFA

Sample name	Determined minimum sample mass (g)	FA content in determined minimum sample mass (nmol FA g ⁻¹ DW soil)	FA content in recommended sample mass (nmol FA g ⁻¹ DW soil)
Vineyard 1	2.0	15.0	16.8
Vineyard 2	2.0	24.8	27.5
Safflower conv	1.0	26.4	27.6
Safflower org	2.0	26.9	29.2
Fallow org	2.0	56.9	52.6
Average	1.8	29.6	30.7

Sample means ($n=2-4$) represent PLFA extraction of five different soils. Below the determined minimum sample mass, total FA content decreased by $\geq 10\%$ from that measured in recommended soil masses (data not shown).

Table 4

Detection limit analysis: minimum sample mass required to obtain mean total FA contents (nmol FA g⁻¹ DW soil) similar to those measured when recommended soil masses were extracted by TSFAME

Sample name	Determined minimum sample mass (mg)	FA content in determined minimum sample mass (nmol FA g ⁻¹ DW soil)	FA content in recommended sample mass (nmol FA g ⁻¹ DW soil)
Vineyard 1	200	157.8	160.8
Vineyard 2	200	186.8	194.3
Safflower conv	200	203.2	203.6
Safflower org	50	178.6	192.5
Fallow org	200	332.0	341.2
Average	170	214.0	218.5

Sample means ($n=2-4$) represent TSFAME extraction of five different soils. Below the determined minimum sample mass, total FA content decreased by $\geq 10\%$ from that measured in recommended soil masses (data not shown).

In addition to CA, we compared total FA content in high mass and low mass samples to determine the minimum sample mass required to extract a total FA content $\geq 90\%$ to that measured in high mass samples (Table 3). These results suggest that ≥ 1.8 g DW soil for PLFA and ≥ 170 mg DW soil for TSFAME should be extracted to obtain reliable total FA contents.

3.2. Sample precision analysis

To illustrate sample-to-sample variability (i.e. sample precision), the CV was compared for the high mass samples for both extraction methods (5 and 8 g, PLFA; 300 and 500 mg, TSFAME) (Table 5). To determine the influence of lower-yielding FAs on sample precision, two FA cutoffs were compared—FAs detected in 50% or more of the samples and FAs detected in 100% of the samples. In all cases (for both cutoffs and for all sample types), there was greater sample-to-sample variability with TSFAME extraction. Coefficients of variation ranged from 1.7 to 4.8-fold

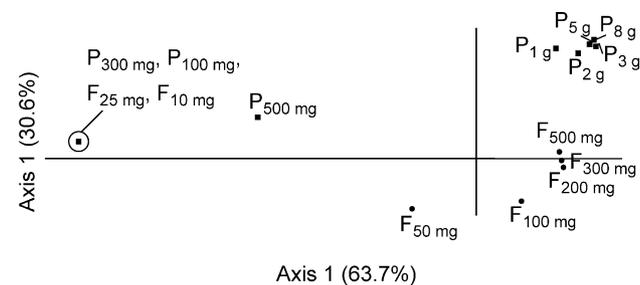


Fig. 1. Detection limit analysis: correspondence analysis of Vineyard 1 soil using either PLFA or TSFAME extraction on decreasing soil masses. PLFA-extracted samples are indicated by the symbol P, and TSFAME-extracted samples are indicated by the symbol F. Decreasing soil mass extracted is indicated by subscript. Fatty acids used in this analysis were present in at least 50% of all Vineyard 1 samples. In total, nine fatty acids were used in this analysis.

Table 5
Mean CV comparing lipid extraction methods

Sample	FA cutoff=50%, 29 FAs		FA cutoff=100%, 23 FAs	
	PLFA	FAME	PLFA	FAME
Vineyard 1	15.7	27.9	15.7	33.8
Vineyard 2	10.4	30.5	10.6	18.1
Safflower conv	12.8	29.7	13.0	21.9
Safflower org	11.4	38.7	4.8	23.0
Fallow org	17.9	66.4	16.4	50.0
Average	13.6	38.6	12.1	29.3

Coefficients of variation were determined only for those FA common to both methods and were based on nanomoles of FA g⁻¹ soil (DW). Two FA cutoffs were used for these calculation—only those FAs detected in at least 50% (FA cutoff=50%) or 100% (FA cutoff=100%) of samples.

higher at the 100% cutoff and 1.8 to 3.7-fold higher with the 50% cutoff in TSFAME versus PLFA extraction. In addition, the CVs were higher at the 50% cutoff compared to the 100% cutoff for TSFAME than for PLFA. For example, comparing the 100 and 50% cutoffs, the mean CV for PLFA-extracted samples increased from 12.1 to 13.6%, whereas the mean CV for TSFAME-extracted samples increased from 29.3 to 38.6%.

3.3. Biomarker comparison

Often, specific biomarker fatty acids or groups of FAs are compared between treatments in experiments using TSFAME or PLFA. Conclusions then are drawn about the enrichment of various microbial groups in different samples. Therefore, the pool sizes (mol% values) of different typical biomarkers (10Me FAs, 18:2 ω 6,9c, and a group of bacterial FAs) were compared between extraction methods within a soil type. For 18 out of 20 comparisons, there were significant differences in the pool sizes between PLFA and TSFAME extracted samples. The biomarkers for actinomycetes (Fig. 2A) and bacteria (Fig. 2B) contributed much larger portions of the total FA pool when soils were extracted with PLFA than with TSFAME. In contrast, in four out of five soil types the fungal biomarker pool (Fig. 2C) was significantly greater in TSFAME samples than in PLFA samples. It also was observed that for four of the five soils TSFAME extracted significantly more short chain FAs (<14 C in length) than PLFA (Fig. 2D). When extracted by PLFA, these FAs were not detected in three of the five soils (Vineyard 1, Vineyard 2, and Safflower org) and made up less than 0.2% of the total FA pool in the Safflower conv and Fallow org samples. In contrast, FAs < 14 C long comprised 1.4–5.5% of the total FA pool when the five soils were extracted with TSFAME.

3.4. Ability to discriminate soils

When plotted together, the PLFA- and TSFAME-extracted soils grouped by method (data not shown), preventing interpretation based on sample type.

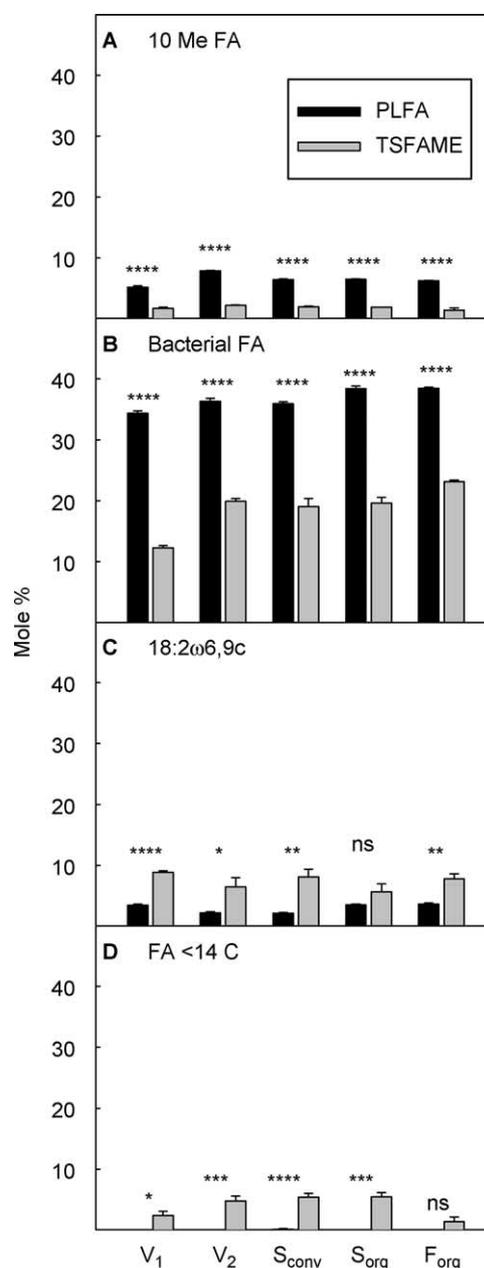


Fig. 2. Univariate ANOVAs comparing PLFA and TSFAME mol percent biomarker values within a soil type. (A) Mol% 10 Me FA, (B) mol% bacterial FA, (C) mol% 18:2 ω 6,9c, (D) mol% FA with chain lengths <14 C. The abbreviation for the five types are as follows: Vineyard 1 (V₁), Vineyard 2 (V₂), Safflower conv (S_{conv}), Safflower org (S_{org}), and Fallow org (F_{org}). Asterisks indicate significant differences between extraction methods within a soil type (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$). Values not significantly different from one another are indicated by 'n.s.'.

Therefore, individual CAs were conducted for each method (Fig. 3A and B).

When analyzed alone, the PLFA-extracted samples clustered based on crop type (Fig. 3A). The almond samples were the most different from other crop types, grouping in the lower left portion of the plot. Although the fig and cotton samples grouped separately from one another, the walnut

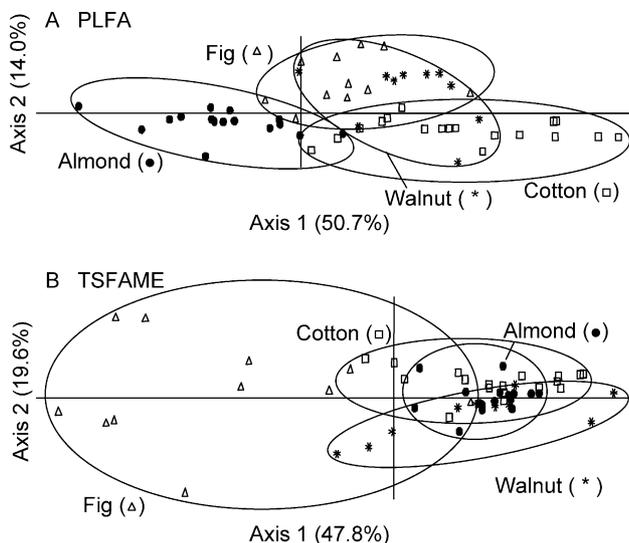


Fig. 3. Correspondence analysis of four agricultural soil samples extracted by PLFA (Fig. 3A) or TSFAME (Fig. 3B). Only those FAs present in all samples within a method were used in this analysis. In total, 24 FAs were used in the analysis of PLFA data, and 21 FAs were used in the analysis of TSFAME data.

samples overlapped with both fig and cotton samples. These three crop types all plotted on the right side of the plot. Overall, the first two axes describe 64.9% of the sample variation based on fatty acid composition.

Although the TSFAME-extracted samples also grouped based on soil type, the underlying plot structure was very different from the PLFA-extracted samples. The almond soils, rather than having a unique FA composition, plotted in the middle of the cotton and walnut samples, suggesting that TSFAME extracted similar lipids from cotton, walnut, and almond soils. In addition, the fig samples, unlike with PLFA extraction, did not group very tightly, indicating a highly variable FA composition based on TSFAME extraction. These samples were widely dispersed and to the left side of the first axis. Overall, the first two axes describe 67.4% of the sample variation based on fatty acid composition.

4. Discussion

4.1. Selection of non-culture based methods

Previously, most microbial methods were culture-based and as such were unsuitable for characterizing entire soil microbial communities. With the advent of non-culture based methods, it became possible to directly extract entire microbial communities from soil. Two popular methods for microbial community analysis are TSFAME and PLFA. As their use increases, it becomes important to determine the appropriate use of each method and to ask the following questions: given the assumptions inherent in each method (1) is one method superior to the other due to possible sampling constraints (sample mass available, time required

for analysis, etc.), (2) does one method provide greater precision, and (3) which method most reliably describes microbial community differences? Our study systematically addressed these questions using a series of agricultural soils which varied by geographical location, soil type, and crop type.

4.2. Detection limit analysis

Overall, TSFAME extraction required significantly less sample mass than PLFA extraction. On average, there was a 10-fold difference in minimum sample mass required by the two methods (≥ 1.1 g PLFA, ≥ 140 mg TSFAME to obtain a reliable community fingerprint; ≥ 1.8 g PLFA, ≥ 170 mg TSFAME to obtain reliable total FA contents) (Fig. 1, Tables 3 and 4). When insufficient soil masses were extracted, an unreliable community fingerprint was obtained by both methods, as only the most dominant FAs were extracted. Therefore, high mass and low mass samples differed in their FA composition, influencing the structure of the CA plots. Although less soil is required for TSFAME extraction, total FA concentrations (nmol FA g^{-1} soil) averaged 7-fold higher in TSFAME samples. Total concentrations likely differed because TSFAME extracts all FAs, including FAs from membranes, storage products, and decomposing plant litter. In contrast, PLFA analysis extracts only phospholipids, which are rapidly degraded following cell death (Pinkart et al., 2002). Although both methods extracted approximately 34 FAs on average, only 29 were common to both methods. These compositional differences are evident in the higher mass samples, with high mass samples grouping by extraction method (Fig. 1).

4.3. Sample precision

Sample-to-sample variability was much lower in PLFA-versus TSFAME-extracted samples, regardless of crop type or FA cutoff (i.e. the number of FAs included in the comparison). Coefficients of variation within sample type were as much as 4.8-fold greater with TSFAME extraction versus PLFA extraction. In addition, when more rare FAs were included (i.e. the 50% cutoff), the CV increased more for TSFAME than PLFA, with an average CV of nearly 40% for TSFAME, compared to approximately 14% for PLFA. The low mean variation of the PLFA-extracted samples is similar to that found by Saetre and Bååth (2000) and Macalady et al. (2000). High sample-to-sample variability (as found in the TSFAME-extracted samples) can lead to decreased ability to discriminate between sample types, as was evident when analyzing the larger soil set (Fig. 2A and B). Although fig samples grouped tightly with PLFA extraction, these samples were much more variable with TSFAME extraction. In fact, the variability within TSFAME fig samples was as great as variability within the other three crop types combined.

4.4. Biomarker analysis

When discussing differences in community composition based on lipid composition, enrichment of specific biomarker FAs often are compared (Zelles, 1999). Using this approach, relative amounts of bacteria, actinomycetes, and fungi can be approximated. However, since PLFA and TSFAME extract lipids from different sources, conclusions based on biomarkers may yield method-specific results. Within our set of five soils, significant differences in pool sizes were detected between PLFA- and TSFAME-extracted samples in 18 out of 20 comparisons (Fig. 2). Interestingly, when soils had been PLFA-extracted, bacterial FA pools were larger, whereas fungal FA pools were smaller. The larger fungal pools that we observed in TSFAME- versus PLFA-extracted samples support Bååth's (2003) observation that the TSFAME- versus method ('total FAME' in his paper) estimates a larger eukaryotic biomass than does PLFA. The higher eukaryotic estimates result from eukaryotes having higher amounts of neutral lipid fatty acids (NLFAs). These NLFAs are detected in TSFAME but not PLFA analysis. In addition to larger fungal pools, in our experiment TSFAME extracted significantly more FAs < 14 C in length. Schutter and Dick (2000) also observed significantly greater pools of short-chain saturated FAMES when soils were TSFAME-extracted. These short chain FAs likely are derived from sources other than living cell membranes, since they were not extracted by PLFA.

4.5. Ability to discriminate soils

Extraction method had the greatest influence on the ability to discriminate soils. The differences due to extraction method were so large in magnitude that differences between soil types could not be discerned when both PLFA- and TSFAME-extracted samples were analyzed together (data not shown). Community composition differentiation by method has been observed between PLFA and substrate utilisation patterns (Biolog) by Bossio and Scow (1998), between TSFAME and ester-linked (EL) FAME by Schutter and Dick (2000), and between total FAME and PLFA by Drijber et al. (2000).

Secondary to extraction method, samples grouped based upon soil type when PLFA- and TSFAME-extracted samples were analyzed separately (Fig. 3). However, the underlying plot structure varied greatly between the two methods. With PLFA extraction, the almond samples were the most different based on their FA composition. In contrast, the fig samples were the most different soil type when extracted using the TSFAME method. In addition, the fig samples were highly variable in their FA composition and, therefore, did not group very tightly.

Although it is obvious that both methods were able to discriminate environmental samples (samples grouped by soil type with both methods), the interpretation of these results varies greatly depending on the extraction method.

Since sample similarity is based upon FA composition, drawing conclusions based on PLFA data is more straightforward, as the sources of PLFAs (i.e. cell membranes) are more discernable than the sources of FAs from TSFAME (i.e. cell membranes, storage products, soil organic matter, etc.). Although some FAs other than those derived from non-microbial sources may be extracted with PLFA methods, Nielsen and Petersen (2000) estimated that this pool is no more than 5–10% of all FAs extracted.

4.6. Conclusions

Overall, PLFA data provide more consistent fatty acid profiles among sample replicates and therefore more reliable sample profiles. PLFA also has an advantage over TSFAME when specifically analyzing microbial community composition. This is because phospholipids are more representative of viable organisms, unlike total fatty acids which include a larger portion of decaying plant material and components of soil organic matter. TSFAME may be advantageous, e.g. when time is a constraint, only very small sample sizes are available, or when it is beneficial to lump differences in organic matter with differences in microbial communities in comparing sites or treatments.

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