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Fatty Acid Methyl Ester Analysis to Identify Sources of Soil in Surface Water

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ABSTRACT

Efforts to improve land-use practices to prevent contamination of surface waters with soil are limited by an inability to identify the primary sources of soil present in these waters. We evaluated the utility of fatty acid methyl ester (FAME) profiles of dry reference soils for multivariate statistical classification of soils collected from surface waters adjacent to agricultural production fields and a wooded riparian zone. Trials that compared approaches to concentrate soil from surface water showed that aluminum sulfate precipitation provided comparable yields to that obtained by vacuum filtration and was more suitable for handling large numbers of samples. Fatty acid methyl ester profiles were developed from reference soils collected from contrasting land uses in different seasons to determine whether specific fatty acids would consistently serve as variables in multivariate statistical analyses to permit reliable classification of soils. We used a Bayesian method and an independent iterative process to select appropriate fatty acids and found that variable selection was strongly impacted by the season during which soil was collected. The apparent seasonal variation in the occurrence of marker fatty acids in FAME profiles from reference soils prevented preparation of a standardized set of variables. Nevertheless, accurate classification of soil in surface water was achieved utilizing fatty acid variables identified in seasonally matched reference soils. Correlation analysis of entire chromatograms and subsequent discriminant analyses utilizing a restricted number of fatty acid variables showed that FAME profiles of soils exposed to the aquatic environment still had utility for classification at least 1 wk after submersion.

Land-use practices that contribute sediment to surface waters have come under increased scrutiny because they impact water quality and aquatic habitats. Where the impact is significant and detrimental, there is a need to develop alternative practices that reduce soil transport. One problem in developing alternative practices is a lack of understanding of where these efforts should be focused. Most watersheds drain regions with multiple land uses and the relative contribution of land-use practices and naturally occurring streambank erosion to sediment transport is unknown.

An approach to identify the predominant source of sediment in surface waters is needed to focus efforts to reduce soil transport. One potential approach, fatty acid methyl ester (FAME) analysis of soil biological communities, exploits the fact that in many cases, combinations of fatty acids isolated from microbial and other organic components of soil provide ''fingerprints'' characteristic of those communities (Kennedy, 1998; Ibekwe and Kennedy, 1999). Interpretation of FAME profiles isolated from environmental samples is complicated by the large number of fatty acids commonly identified and by the fact that environmental conditions impact the nature of lipid components that constitute soil biological communities (Marr and Ingraham, 1962; Harwood and Russell, 1984; Tunlid and White, 1992; Frostegård et al., 1993). Multivariate statistical methods provide capabilities to discriminate between FAME profiles that represent contributions of neutral lipid fatty acids, glycolipid fatty acids, and lipopolysaccharide 3-OH and 2-OH fatty acids from decaying plant material as well as viable and nonviable microorganisms. This discrimination is conducted without knowledge of the source of the fatty acids or taxonomy of microbial components that contribute to the profile. When more specific knowledge of microbial community composition is required, the use of phospholipid fatty acid profiles is more appropriate because they are representative of viable microorganism communities (Zelles et al., 1992).

Fatty acid analyses have been used to monitor changes in aquatic biofilms (Guckert et al., 1992; Scholz and Boon, 1993), and to characterize ground water communities (Glucksman et al., 2000). The approach has not been used to track the fate of sediment transported to the aquatic environment and consequently, the impact of aquatic microflora on soil FAME profiles after transport to the aquatic environment is not known.

We previously used a Bayesian routine to select fatty acid variables for discriminant analysis of FAME profiles to differentiate agricultural soils subject to contrasting tillage and ground cover practices (Dierksen et al., 2002). Subsequent efforts to apply the approach to identify sediments in surface waters demonstrated that the fatty acid variables useful in the previous study were not appropriate for classifying similar, but distinct soil types collected during different seasons of the year. These findings were consistent with previous reports that fatty acid composition of cells is affected by environmental conditions that impact substrate availability (Marr and Ingraham, 1962; Bossio et al., 1998). The objective of this research was to identify fatty acids suitable for classification of contrasting reference soils by multivariate statistical analyses and to determine whether these variables were suitable for analysis of soil FAME profiles collected in different months. We also evaluated

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Abbreviations: FAME, fatty acid methyl ester.

the stability of FAME profiles after reference soils were submerged in the aquatic environment.

MATERIALS AND METHODS

Soil Sample Collection

Soil samples were collected at specified depths up to 10 cm using a 2.54-cm-diameter soil corer. The corer was cleaned between samples with water followed by methanol and samples were placed in zip-lock plastic bags and stored for no longer than 7 d at 6°C.

Concentration of Suspended Particulates

Vacuum filtration and aluminum sulfate precipitation were compared for their effectiveness in collection of suspended particulates. Vacuum filtration was used to pass samples through a Super 450 (0.45 μm) membrane (Gelman Scientific, Ann Arbor, MI), fitted into an acid (1% HCl) washed filter holder. The membrane was dried overnight at 22°C, cut into strips, placed into a 15-mL Pyrex screw cap tube, and stored at 6°C until subsequent extraction of fatty acids for FAME analysis.

Aluminum sulfate precipitation was performed by adding 160 μL of 48% $Al(SO_4)_3$ per mL of sample. Immediately afterward, 5 *M* NaOH was added to a concentration of 160 μL of per mL of sample. The mixture was shaken and centrifuged for 15 min at 3000 × *g* and the pellet was transferred to a clean 15-mL Pyrex glass tube. After final centrifugation at 1000 × *g* for 15 min, the supernatant was discarded and the pellet stored at 6°C until subsequent extraction of fatty acids for FAME analysis.

Comparison of Particulate Collection Approaches for *Pseudomonas fluorescens*

Cultures of *Pseudomonas fluorescens* were used to provide a uniform source of FAMEs from which to evaluate particulate collection approaches. Bacteria were propagated in pseudomonas minimal salts medium (Bolton et al., 1989) at 27°C on a rotary shaker (250 rpm). After the cultures reached stationary phase (5 d), 250 mL of the broth were centrifuged at 3000 × *g* for 15 min, the supernatant was discarded, and the pellet resuspended in 250 mL of dH_2O. Eight 10-mL aliquots were transferred to 50-mL glass centrifuge tubes, centrifuged at 3000 × *g*, and the supernatant discarded. Six pellets were resuspended in 25 mL dH_2O and three were used for aluminum sulfate precipitation, the other three for membrane filtration. The remaining two pellets were extracted directly in the glass centrifuge tubes.

Water was collected from the confluence of a ditch draining a perennial ryegrass (*Lolium perenne* L.) seed production field and the Calapooia River following a heavy rain event in March 2000. Four 250-mL aliquots of sediment-containing water were used for particulate trapping by membrane filtration and four used for aluminum sulfate precipitation.

Comparison of Methods to Trap Suspended Particulates from Water Samples

Soil was collected from the surface of the confluence of drainage from a perennial ryegrass seed production field (44°31′59″ N, 123°08′31″ W). The moisture content of the soil was determined and soil added to 300 mL dH_2O to a concentration of 8 g soil dry wt./L. The soil suspension was mixed and aliquots removed and diluted with dH_2O to give eight replicates with concentrations of 8 g soil/L, 2 g soil/L, 1.2 g soil/L, and 0.28 g soil/L. Four 25-mL aliquots of each concentration were used for particulate trapping by membrane filtration, the remaining four used for aluminum sulfate precipitation. Four 1-g (dry wt.) samples of the ditch surface soil also were extracted and analyzed for FAMEs.

Comparison of Fatty Acid Methyl Esters from Contrasting Soils Adjacent to Surface Water

Soil cores 2.4 cm in diameter were collected to a depth of 10 cm at six locations in the mid-Willamette Valley of Oregon (44°31′59″ N, 123°08′31″ W), in May 2000, along a 350-m transect of contrasting land use conditions, from an agricultural field through a forested riparian area, and to the Calapooia River (Fig. 1). The specific sampling locations were (i) a fallow perennial ryegrass seed production field (44°32′00″ N, 123°08′29″ W), (ii) a field drainage location as it enters a forested riparian area (44°31′58″ N, 123°08′28″ W), (iii) a forested riparian area (44°31′55″ N, 123°08′31″ W) and (iv) its drainage to the Calapooia River channel (44°31′55″ N, 123°08′36″ W), (v) surface sediment at the river's edge (44°31′55″ N, 123°08′36″ W), and (vi) a cut bank on the opposite side of the river (44°31′55″ N, 123°08′37″ W). The agricultural field soil type was a Waldo (fine, mixed, mesic Fluvaquentic Haplaquollis) and the forested riparian soil was a Chehalis (fine-silty, mixed, mesic Cumulic Ultic Haploxerolls). Four surface water samples draining the riparian area were also collected after a precipitation event to serve as a source of transported sediment for application of FAME analysis in sediment classification.

Determination of Fatty Acid Methyl Ester Stability in the Aquatic Environment

Two independent experiments were conducted to determine the stability of soil FAMEs in the aquatic environment.

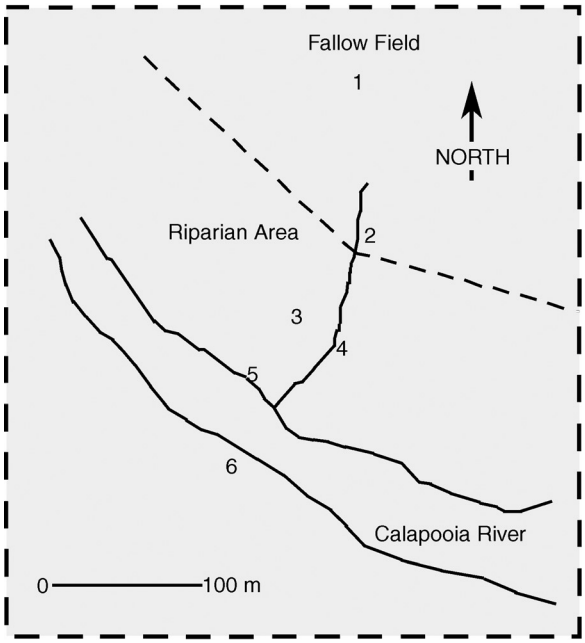

Fig. 1. Specific sampling locations near the Calapooia River, Willamette Valley, Oregon. (1) A fallow perennial ryegrass seed production field (44°32′02″ N, 123°08′29″ W). (2) A field drainage location as it enters a forested riparian area (44°31′58″ N, 123°08′28″ W). (3) A forested riparian area (44°31′55″ N, 123°08′31″ W) and (4) its drainage to the Calapooia River channel (44°31′55″ N, 123°08′36″ W). (5) Surface sediment at the river's edge (44°31′55″ N, 123°08′36″ W). (6) A cut bank on the opposite side of the river (44°31′55″ N, 123°08′37″ W).

In both trials, reference soils were submersed into waterways, and FAME profiles were determined and compared to those developed from the soils before submergence. The first experiment was designed to compare the stability of FAMEs when soils from contrasting land uses were submerged in river water. Multiple 10-cm cores of soil were collected in June 2000 from an established tall fescue (*Festuca arundinacea* Schreb.) seed production field (44°38′01″ N, 123°12′05″ W) (Woodburn soil; fine-silty, mixed, superactive, mesic Aquultic Argixeroll), a mixed hardwood–conifer riparian zone (44°31′57″ N, 123°08′33″ W) (Chehalis soil) adjacent to the Calapooia River, and a conifer stand (Woodburn soil) adjacent to grass seed fields in the mid-Willamette Valley (44°37′51″ N, 123°11′44″ W). Samples submerged in Oak Creek (Benton County, OR). The cores from each soil type were mixed and ten 1.0-g (dry wt.) samples from each soil were placed into 15-mL polypropylene centrifuge tubes and placed into 2.54- × 45-cm PVC pipe that was capped on the bottom. The holder was perforated to allow free movement of water through the assembly over the surface of the submerged soil and attached to a steel pipe, which was driven into the streambed to submerge the soil samples to a depth of approximately 60 cm. Replicated samples of each soil type were removed at weekly intervals for FAME analysis and compared with those obtained from matched samples of nonsubmerged soil. Blank tubes were included as extraction controls to account for the possible extraction of fatty acids from the plastic tubes. Discriminant analyses were used to evaluate the impact of the aquatic environment on FAME profiles.

In the second experiment, 5-g (dry wt.) soil samples from 10-cm cores were collected in May 2000 from a fallow grass seed production field (44°32′02″ N, 123°08′29″ W; Waldo soil) and an adjacent mixed hard and softwood riparian zone (44°31′57″ N, 123°08′33″ W; Chehalis soil). The soil samples were placed in open 15-mL plastic centrifuge tubes which in turn were placed inside of a 2.54- × 45-cm section of PVC pipe capped on the bottom. The holder was perforated to allow free movement of water through the assembly over the surface of the submerged soil and the assembly was attached to a steel rod, which was driven into the streambed of the Calapooia River (44°31′56″ N, 123°08′35″ W) (Linn County) in western Oregon. The assembly submerged the soil samples to a depth of approximately 60 cm. At weekly intervals, 1-g samples were removed from the assembly, FAME analyses were conducted, and chromatograms representing dry and submerged soils were compared using correlation analysis. Coefficients of correlation were conducted by executing the ''cor'' function in R, Version 1.1.1 on a data matrix where columns contained all individual fatty acids identified by MIDI Sherlock Microbial Identification System software (Version 3.0) (MIDI, 1999) while rows contained the percentage of each fatty acid present in individual samples.

Fatty Acid Methyl Ester Procedure

Fatty acid methyl esters were extracted from all samples as described by Kennedy (1994). Briefly, each sample was saponified for 30 min at 100°C in 3.75 *M* NaOH in 50% methanol, methylated for 10 min at 80°C by addition of 6 *M* HCl in methanol, extracted into two 1-mL aliquots of hexane and MTBE (1:1), and the fractions combined and washed in 0.3 *M* NaOH before concentration by overnight volatilization of the hexane and MTBE. Hexane and MTBE (150 μL per g soil sample and 75 μL for suspended particulate) were added to the volatilized vials and transferred to 200-μL inserts in 2-mL crimp top vials. The extracted samples were analyzed on a HP 5890 Series 2 gas chromatograph (Hewlett-Packard, Palo Alto, CA) using an Agilent Technologies (Palo Alto, CA) ultra 2 cross-linked 5% PH ME siloxane capillary column, 25 m long × 0.20 mm. The instrument was programmed and controlled with MIDI Sherlock Microbial Identification System software (Version 3.0) (MIDI, 1999) using MIDI microbial calibration standards (#1 quantitative saturated nC:9 to nC20:0 plus 2 and 3 Hydroxy). Fatty acid methyl esters were quantified based on these calibration standards. Fatty acids are denoted using MIDI nomenclature and were designated by the number of carbon atoms, a colon followed by the number of double bonds, and then the position of the first double bond from the ω end of the molecule. *Cis*- and *trans*- isomers are indicated by c or t. An α character following the designations indicates a unique fatty acid in which the location of the double bond was not determined by the MIDI software. ''Summed features'' indicates overlapping peaks for more than one identified fatty acid at a particular retention time. For example, the summed feature 16:1 ω7/15 iso 2OH peak cannot distinguish 16:1 ω7 from 15 iso 2OH. Unknown peaks were listed by retention time only.

Data Acquisition and Analysis

Chromatogram peaks representing FAMEs that co-eluted with authentic reference standards were quantified by the Sherlock Microbial Identification System software (Version 3.0) (MIDI, 1999) as percentage of total fatty acids. Subsets of FAMEs commonly associated with bacterial and fungal populations including straight chain saturated and monounsaturated fatty acids (12:0, 15:0, 17:0, 17:1 ω7c), 10- methyl-branched iso- and anteiso-fatty acids (13:0 iso, 14:0 anteiso, and 19:0 10 methyl), hydroxylated fatty acids (10:0 3OH, 11:O 3OH, and 15:0 iso 3OH), cyclopropylated (17:0 cyclo), and 16- or 18-carbon fatty acids (16:0, 18:0, and 18:1 ω7c) were selected by visual inspection for use in discriminant analyses. This subset was restricted to those FAMEs that were detected in at least two reference land use categories. An iterative process was used to distinguish combinations of these 14 FAMEs that provided the most accurate classification of reference soils in discriminate analyses (SPSS Version 13.0; SPSS, 2004). The visual inspection included selection of variables by examining computer-generated rapid overlays of FAME profiles and noting peaks that were commonly associated with, or that appeared to distinguish specific soil types.

We applied both nonsupervised classification (principle components analysis) and supervised classification (discriminant analysis). The clusters generated by the use of principle components analysis allowed us to infer a structure for the data with no prior assumptions about that structure. In this case, the intent was to evaluate whether soil categories could be inferred from principle components analysis of FAME profiles without use of the known categories. In contrast, our discriminant analyses used prior knowledge of soil categories to construct and test a model of classification. Discriminate analyses utilizing FAMEs selected by a Bayesian variable selection previously described (Dierksen et al., 2002) also were conducted for comparative purposes. Principle component analysis was conducted utilizing R and the MASS package for R which is based on Venables and Ripley (2002). Both software packages are available online at http://www.stats.ox.ac.uk/pub/MASS4/Software.html (verified 27 Sept. 2005).

The error rates were calculated using 10-fold cross-validation (Lachenbruch and Mickey, 1968; Stone, 1974). To accomplish this, FAME datasets were divided into 10 subsets of equal size. Ten sets of discriminant analyses were conducted on the data, deleting a different subset in each analysis. The error rate represented an average calculated from this set of analyses.

RESULTS

Comparison of Particulate Collection Methods

The first technical challenge in application of FAME analysis to suspended sediment was concentration of particles from relatively dilute suspensions. We evaluated vacuum filtration and aluminum sulfate precipitation as potential approaches to concentrate bacterial cells from a pure culture filtrate and to concentrate sediment from water. In practice, aluminum sulfate concentration was more rapid than filtration and fatty acid profiles developed from bacterial cells collected by both approaches were similar except for the apparent enhanced recovery of 14:0 and 18:0 by filtration (Table 1). Control trials utilizing both methods on bacteriological media or water containing no sediment demonstrated that neither approach contributed background signal to the FAME analysis (data not shown). A separate experiment utilizing sediment from river water collected by both methods confirmed that FAME recovery was similar using either approach (data not shown). A separate comparison utilizing runoff water from an agricultural drainage confirmed that FAME recovery was similar using either approach (Table 2). Subsequent analyses were performed using aluminum sulfate precipitation.

Comparison of Fatty Acid Methyl Esters from Contrasting Soils Adjacent to Surface Water

To confirm that our approach had the capability to distinguish contrasting reference soils and to determine the utility of fatty acids previously identified as useful variables in discriminant analysis, we prepared FAME chromatograms of soils from six apparent land uses which identified a total of 108 FAMEs. The set of three fatty acids (16:0 10 methyl, 17:1 ω7c, and 18:0) that proved useful as variables in multivariate statistical analyses in our previous study (Dierksen et al., 2002) were not applicable in these studies because the first two fatty acids were not present in all chromatograms of these soils. By visual inspection we identified three other fatty acids (10:0 3OH, 17:1 ω7c, and 19:0 10 methyl) that appeared to account for major differences among reference soil types. Use of these FAMEs as variables in discriminant analyses to classify replicate samples of reference soils provided an 11.1% error rate (Table 3). Principle component analysis using these FAMEs showed a mixed resolution of soils representing contrasting land uses (Fig. 2). Soil FAMEs from the agricultural field (A in Fig. 2) and a drainage ditch within the same field (B in Fig. 2) clustered together and could not be separated. In contrast, FAMEs from soil in the adjacent riparian zone (C in Fig. 2) clustered separately from A and B. Soil FAMEs from the drainage ditch within the riparian zone (E in Fig. 2) appeared as an apparent cluster as did soil FAMEs collected from the stream bed (E in Fig. 2). Fatty acid methyl esters from soil collected from the opposite stream bank under a wooded

Table 1. Comparison of fatty acid methyl esters (FAMEs) isolated from *Pseudomonas fluorescens* using filtration and aluminum sulfate precipitation.

	Pellet†	Filter membrane‡		Precipitation§	
FAME	Rep 1	Rep 1	Rep 2	Rep 1	Rep 2
	%				
Saturated					
10:0	ND¶	0.12	ND	0.17	0.14
12:0	4.36	4.49	4.47	5.02	3.63
14:0	ND	1.64	1.65	ND	0.81
16:0	39.26	37.80	38.21	38.40	39.69
18:0	0.72	2.65	2.59	0.81	0.81
Unsaturated					
16:1 ω7c/15 iso 2OH	9.98	10.03	9.94	10.91	10.81
17:1 ω7c	ND	0.30	ND	ND	0.27
18:1 ω7c	ND	9.29	9.38	9.67	10.43
Hydroxy					
8:0 3OH	0.04	ND	0.08	ND	ND
10:0 3OH	3.13	2.61	3.45	3.56	2.37
12:0 2OH	2.36	2.38	2.36	2.66	2.06
12:0 3OH	4.59	3.88	3.68	2.16	2.87
17:0 iso 3OH	0.25	0.39	ND	ND	ND
Cyclo					
17:0 cyclo	21.23	21.63	22.36	24.18	24.90
19:0 cyclo ω8c	ND	2.10	1.65	ND	1.85

† Bacterial cells collected by centrifugation from pure culture.
‡ Bacterial cells were concentrated from culture medium by 0.45-μm membrane filtration.
§ Bacterial cells were concentrated from culture medium by aluminum sulfate precipitation.
¶ Not determined.

Table 2. Comparison of fatty acid methyl esters (FAMEs) isolated from soil particulate in water from an agricultural drainage using filtration and aluminum sulfate precipitation.

FAME	Filter membrane†	Precipitation‡
	%§	
Saturated		
11:0	0.15	0.07
11:0 anteiso	0.10	0.10
12:0	4.94	3.84
12:0 anteiso	0.95	0.71
13:0	0.22	ND¶
13:0 iso	0.41	0.30
14:0 anteiso	64.36	64.90
15:0 anteiso	0.19	ND
16:0	0.66	ND
17:0	9.42	8.51
17:0 anteiso	0.73	1.09
18:0	0.20	0.19
19:0	0.13	ND
Unsaturated		
13:1	0.22	ND
15:1 ω5c	0.19	ND
15:1 ω8c	0.89	0.83
16:1 ω5c	0.43	ND
16:1 ω11c	1.48	0.80
16:1 iso	0.10	ND
17:1 ω6c	0.17	0.34
17:1 ω7c	0.47	0.74
18:1 ω5c	0.41	0.39
18:1 iso	0.89	1.27
Hydroxy		
10:0 3OH	0.77	0.62
11:0 2OH	1.02	1.28
12:0 3OH	0.33	0.19
13:0 2OH	1.74	1.62
15:0 2OH	0.09	ND
15:0 iso 3OH		
17:0 iso 3OH	0.39	ND
Cyclo		
17:0 cyclo	0.40	ND
19:0 cyclo ω8c	0.29	0.25

† Particulate concentrated from water sample by 0.45-μm membrane filtration.
‡ Particulate concentrated from water sample by aluminum sulfate precipitation.
§ Percentage of total named fatty acids in Sherlock Microbial Identification System software (Version 3.0) (MIDI, 1999).
¶ Not determined.

Table 3. Quadratic discriminant analysis of fatty acid methyl ester (FAME) profiles to classify reference soils from an agricultural landscape utilizing variables selected by visual inspection.†

	Discriminant analysis classification					
Soil sample	**Fallow**	**Ditch**	**Adjacent**	**Draining**	**Bottom**	**Bank**
Fallow field	***4***	**1**	**0**	**0**	**0**	**1**
Ditch draining field	**1**	***4***	**0**	**0**	**0**	**1**
Adjacent riparian zone	**0**	**0**	***6***	**0**	**0**	**0**
Ditch draining riparian zone	**0**	**0**	**0**	***6***	**0**	**0**
Bottom sediment − adjacent stream	**0**	**0**	**0**	**0**	***6***	**0**
Bank of adjacent stream	**0**	**0**	**0**	**0**	**0**	***6***
Error rate = 11.1%						

† Quadratic discriminant analysis was used to classify FAME profiles developed from contrasting soils into data classes developed from replicated FAME analyses. Numbers in italic type represent correct classifications.

riparian zone (F in Fig. 2) clustered closest to agricultural soils but were not readily distinguished. The discriminant analyses (Table 3) clearly distinguished the soil classes identified as A and B in Fig. 2, two soils that were expected to be very similar.

Determination of Fatty Acid Methyl Ester Stability in the Aquatic Environment

To determine the impact of the aquatic environment on the stability of soil FAME profiles, FAMEs from dry reference soils collected in December 2000 were compared with those obtained after reference soils were submerged in Oak Creek for specified periods of time. Using the previously described Bayesian variable selection approach, we identified two fatty acids (18:1 ω7c and 17:1 ω7c) for classifications based on differences between reference soil chromatograms. The dry soils were easily differentiated (error rate = 4.6%) including those representing the interface of the agricultural field with the riparian zone where differences were expected to be slight (Table 4). Submergence of reference soils for

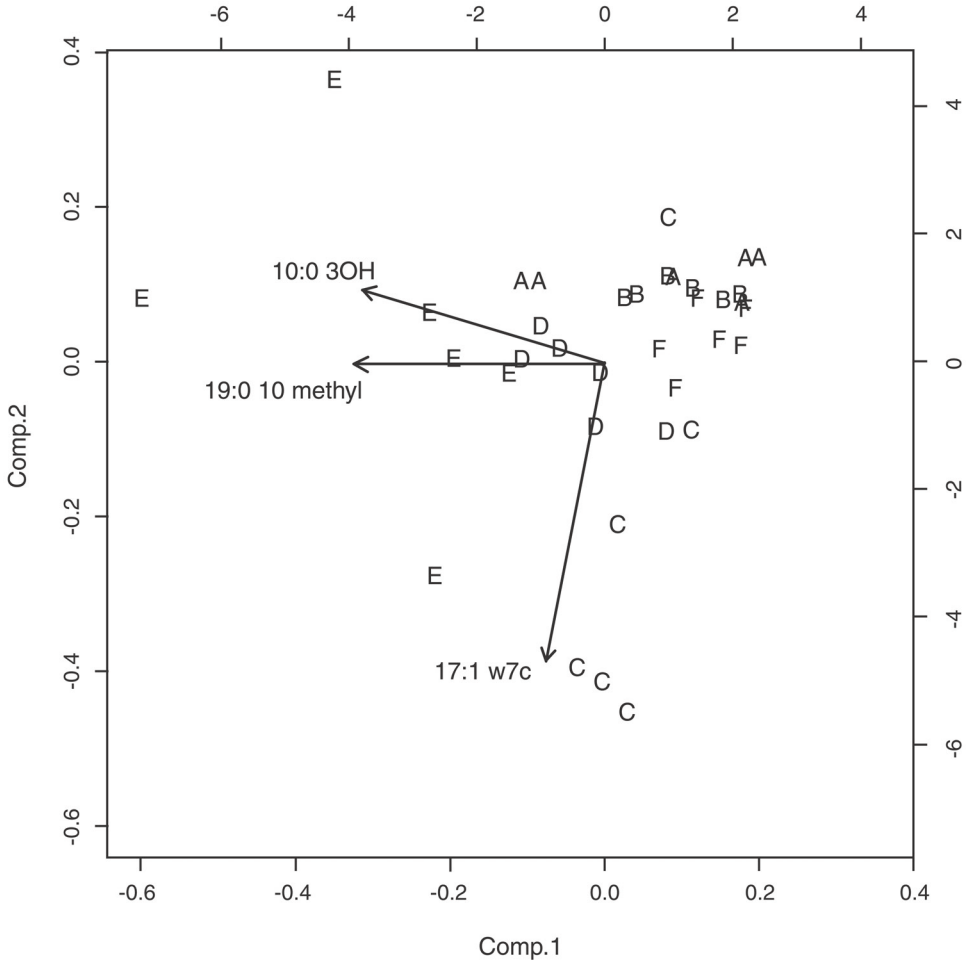

Fig. 2. Principle component analysis of fatty acid methyl esters (FAMEs) extracted from six contrasting soil sites within an agricultural landscape. A = soil from fallow field; B = soil from ditch draining field; C = soil from adjacent riparian zone; D = soil from ditch draining riparian zone; E = soil from bottom sediment from adjacent stream; F = soil from bank of adjacent stream. Proportion of variance, Principle Components 1, 2, and 3: 0.44, 0.33, 0.23.

Table 4. Utility of fatty acid methyl ester (FAME) profiles to classify reference soils from an agricultural landscape utilizing variables selected by a Bayesian approach.†

	Discriminant analysis classification		
Soil sample land use	Field drainage	Agricultural	Wooded riparian
Field drainage at entrance to wooded riparian zone	*7*	1	0
Agricultural field	0	*6*	0
Wooded riparian zone	0	0	*8*
Error rate = 4.6%			

† Quadratic discriminant analysis was conducted to classify FAME profiles developed from soil samples from selected land uses that were submerged in an aquatic environment into data classes developed from replicated FAME analyses of nonsubmerged soils representing contrasting land uses. Numbers in italic type represent correct predictions.

Table 5. Stability of fatty acid methyl ester (FAME) profiles from reference soils submerged in an aquatic environment for 7 d.†

	Discriminant analysis classification		
Soil sample land use	Field drainage	Agricultural	Wooded riparian
Field drainage at entrance to wooded riparian zone	*4*	1	0
Agricultural field	0	*4*	0
Wooded riparian zone	2	0	*7*
Error rate = 16.7%			

† Quadratic discriminant analysis was conducted to classify FAME profiles developed from soil samples from selected land uses that were submerged in an aquatic environment into data classes developed from replicated FAME analyses of nonsubmerged soils representing contrasting land uses. Numbers in italic type represent correct predictions.

Table 6. Stability of fatty acid methyl ester (FAME) profiles from reference soils submerged in an aquatic environment for 14 d.†

	Discriminant analysis classification		
Soil sample land use	Field drainage	Agricultural	Wooded riparian
Field drainage at entrance to wooded riparian zone	*7*	1	1
Agricultural field	0	*6*	2
Wooded riparian zone	0	0	*4*
Error rate = 19.1%			

† Quadratic discriminant analysis was conducted to classify FAME profiles developed from soil samples from selected land uses that were submerged in an aquatic environment into data classes developed from replicated FAME analyses of nonsubmerged soils representing contrasting land uses. Numbers in italic type represent correct predictions.

7 d altered FAME profiles sufficiently to increase the error rate to 16.7% (Table 5) and the most frequent errors involved distinguishing the two soil types that were expected to be most similar. Soil from the riparian zone was distinguishable from that originating in the agricultural field. After 14 d of submergence, the error rate was 19.1% when FAME profiles from the wooded riparian zone were more difficult to distinguish from field soils (Table 6).

Our discriminant analyses used to compare FAME profiles relied on a restricted number of fatty acid variables. A contrasting approach, correlation analysis, was applied that used all fatty acids in the FAME chromatograms to evaluate the stability of fatty acid profiles in the second study conducted in the Calapooia River (Table 7). Reference soil from the agricultural field was distinct from soil collected from the adjacent riparian zone, and FAME profiles of the reference soil had high correlation coefficients with dry reference soil through at least 2 wk of submergence. After 3 wk of submersion, chromatograms developed from the agricultural soil had significantly greater correlation with those developed from submerged riparian soil.

DISCUSSION

Analysis of fatty acid profiles developed from FAMEs or phospholipid fatty acids (PLFAs) has been applied to characterize biological communities in a broad range of terrestrial systems (Cavigelli et al., 1995; Kennedy and Busacca, 1995; Zelles et al., 1995; Buyer and Drinkwater, 1997; Bossio et al., 1998; Drijber et al., 2000; Ritchie et al., 2000; Dierksen et al., 2002; Madan et al., 2002; Schutter and Dick, 2002) and aquatic systems (Guckert et al., 1992; Scholz and Boon, 1993; Glucksman et al., 2000), but the utility of FAMEs or PLFAs to track transport of soil to aquatic systems has not been determined. The general approach involves use of multivariate statistical analyses to compare fatty acid profiles of references with those prepared from sampled material. Although fatty acid composition of bacteria propagated in pure culture under defined conditions is highly reproducible and permits construction of diagnostic ''libraries,'' fatty acids present in soils are highly affected

Table 7. Correlation analysis of chromatograms comparing fatty acid methyl esters (FAMEs) isolated from agricultural and riparian reference soils that were submerged in a riverine environment for up to 5 wk.†

	Correlation coefficient							
	Agricultural soil						Riparian soil	
	0 wk	1 wk	2 wk	3 wk	4 wk	5 wk	1 wk	2 wk
0 wk	1.00	0.98	0.96	0.078	0.093	0.088	0.039	0.069
1 wk	NA‡	1.00	0.94	0.83	0.91	0.89	0.44	0.75
2 wk	NA	NA	1.00	0.79	0.94	0.94	0.41	0.73
3 wk	NA	NA	NA	1.00	0.75	0.83	0.62	0.88
4 wk	NA	NA	NA	NA	1.00	0.87	0.36	0.67
5 wk	NA	NA	NA	NA	NA	1.00	0.41	0.72
Riparian 1 wk	NA	NA	NA	NA	NA	NA	1.00	0.84
Riparian 2 wk	NA	NA	NA	NA	NA	NA	NA	1.00

† Correlation analyses were performed on chromatograms to evaluate whether FAME profiles would have less utility for identifying the source of soil after submersion in surface water for specified lengths of time. Reference soils from agricultural and riparian land uses were submerged in the Calapooia River (Linn County, OR) for 5 and 2 wk, respectively, sampled weekly for FAME analysis, and FAME profiles from submerged soils were compared to those developed from nonsubmerged reference soils.

‡ Not available.

by the season, farming operations, and environmental conditions (Bossio et al., 1998). This is especially true utilizing the extraction technique we chose to recover fatty acids derived from the entire soil biological community including microbial populations, decaying plant tissue, and other soil organic components. We reasoned that inclusion of FAME contributions from all sources provided information representative of contrasting land-use impacts. In addition, FAMEs tend to be more stable in the environment, a characteristic we desired for identification of soil after transport to surface waters. We did not compare FAME profiles with phospholipid fatty acid profiles to quantify contributions from viable microorganisms. Our results suggest that the use of FAME analysis to identify soil in surface water requires preparation of reference soil data classes that match the predominant environmental conditions during which sediment transport occurs. Caution in interpreting FAME data is needed because it is unlikely that fatty acid profiles will be representative of any soil type over long periods of time. Seasonal changes have also been demonstrated in phospholipid fatty acid profiles developed from lake sediments (Smoot and Findlay, 2001), marine benthic communities (Findlay and Watling, 1998), and riverine microbial communities (Langworthy et al., 1998).

The apparent seasonal changes in soil fatty acid composition we observed were likely responsible for the continued need for variable selection. The combination of three fatty acids (16:0 10 methyl, 17:1 ω7c, and 18:0) previously identified by a Bayesian variable selection approach (Dierksen et al., 2002) provided 19.4% error rate in classifying reference soils collected in May 2000 in this study, resolution we judged inadequate. Visual inspection of chromatograms suggested fourteen fatty acids had potential for use as variables and included straight chained saturated and mono-unsaturated fatty acids (12:0, 15:0, 17:0, 17:1 ω7c), methyl-branched fatty acids of the iso- and anteiso- configurations predominant in Gram-positive bacteria (13:0 iso, 14:0 anteiso, and the methylated 19:0 10 methyl), hydroxylated fatty acids common in Gram-negative bacteria (10:0 3OH, 11:O 3OH, and 15:0 iso 3OH), cyclopropylated fatty acids common to Gram-negative bacteria (17:0 cyclo), and 16- or 18-carbon fatty acids (16:0, 18:0, and 18:1 ω7c) that are widespread in nature. Stepwise discriminant analyses determined that nine (Table 2) reduced the error rate to less than 3% and provided clear classification of four replicate water samples collected as "runoff" from the riparian zone during a spring rainfall.

Different sets of fatty acids were detected in reference soils collected in June and December 2000 that were used to determine the stability of soil FAME profiles after submergence. While there was considerable overlap among the type of fatty acids detected, not all previously used fatty acid variables were present in these soils. Variable selection is the critical component of developing strategies to classify sediments into soil data classes. Correlation analysis utilizing the entire chromatograms of the second set of submerged soils suggested greater stability of FAME profiles than indicated by discriminant analyses which utilized a restricted variable set. Although correlation analysis is not a classification tool, the high correlation of FAME chromatograms from submerged soils with that of dry reference soils suggests that further efforts to improve variable selection would enhance the resolution of classification approaches.

These data indicate that successful classification of sediments on the basis of FAME analysis is possible provided soil FAME profiles are developed for reference soils collected at the same time as surface water samples. The FAME profiles are stable for 1 to 2 wk after soil is transported to surface water so it is not critical that water is collected immediately after a precipitation event. After 2 wk, both discriminant and correlation analyses of FAME chromatograms suggested that fatty acid compositions of contrasting reference soils immersed in water became more similar and consequently, more difficult to classify. Whether this resulted from fatty acid degradation in the aquatic environment or colonization of sediment by aquatic microflora with distinct fatty acid composition is not known.

CONCLUSIONS

Fatty acid methyl ester analysis holds promise for identifying the primary source of soil in surface waters. Classification of FAME profiles developed from sediment will need to utilize FAME data classes developed from reference soils collected during the season and under the environmental conditions expected during transport of the soil to surface waters. Successful multivariate analysis of the FAME datasets will rely on identification of appropriate fatty acids for use as variables, and these variables need to be identified in each new set of reference soils.

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