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Accuracy, Reproducibility, and Interpretation of Fatty Acid Methyl Ester Profiles of Model Bacterial Communities†

SHERIDAN KIDD HAACK,[1]* HELEN GARCHOW,[1,2] DAVID A. ODELSON,[1,3] LARRY J. FORNEY,[1,4] AND MICHAEL J. KLUG[1,2,4]

Center for Microbial Ecology,[1] Department of Microbiology,[4] and Kellogg Biological Station,[2] Michigan State University, East Lansing, Michigan 48824, and Department of Biology, Central Michigan University, Mt. Pleasant, Michigan 48859[3]

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We determined the accuracy and reproducibility of whole-community fatty acid methyl ester (FAME) analysis with two model bacterial communities differing in composition by using the Microbial ID, Inc. (MIDI), system. The biomass, taxonomic structure, and expected MIDI-FAME profiles under a variety of environmental conditions were known for these model communities a priori. Not all members of each community could be detected in the composite profile because of lack of fatty acid "signatures" in some isolates or because of variations (approximately fivefold) in fatty acid yield across taxa. MIDI-FAME profiles of replicate subsamples of a given community were similar in terms of fatty acid yield per unit of community dry weight and relative proportions of specific fatty acids. Principal-components analysis (PCA) of MIDI-FAME profiles resulted in a clear separation of the two different communities and a clustering of replicates of each community from two separate experiments on the first PCA axis. The first PCA axis accounted for 57.1% of the variance in the data and was correlated with fatty acids that varied significantly between communities and reflected the underlying community taxonomic structure. On the basis of our data, community fatty acid profiles can be used to assess the relative similarities and differences of microbial communities that differ in taxonomic composition. However, detailed interpretation of community fatty acid profiles in terms of biomass or community taxonomic composition must be viewed with caution until our knowledge of the quantitative and qualitative distribution of fatty acids over a wide variety of taxa and the effects of growth conditions on fatty acid profiles is more extensive.

Profiles of fatty acids derived from phospholipid components of the cellular membranes of microorganisms have been used to estimate microbial biomass and provide insight into the taxonomic and functional diversity and nutritional status of microorganisms associated with sediments (2, 4, 8, 11, 12, 19, 22, 24, 27), subsurface environments (28), soils (1, 35), stream epilithic biofilms (14, 27), bioreactors (20, 21), and plant roots (31, 32). In addition, fatty acid profiles have been used to distinguish between soil communities subjected to different agricultural practices (35), to pollution (1), or to addition of compost media (32), rhizosphere communities under different soil treatments (31, 32), stream epilithon communities at different spatial locations along a stream gradient (14) or at different times of the year (27), and sediment communities along a spatial gradient (11).

Investigators have taken a variety of approaches to the interpretation of community fatty acid profiles. These have included tabulation of fatty acids known or presumed to be unique to a given taxonomic group or comparisons of profiles on the basis of within-profile ratios of groups of fatty acids (e.g., see references 9, 11, 19, 24, 27, and 32) or use of multivariate statistics to discriminate between composite profiles (1, 8, 14, 24, 27, 28). Regardless of the approach, interpretations of community level fatty acid profiles have been based on the assumptions that phospholipids make up a relatively constant proportion of the cell biomass and that fatty acid variation between taxonomic groups results in "signatures" which can be detected, and unambiguously interpreted, in community level profiles (33, 34). However, phospholipid contents of microorganisms vary across taxa (18), and although fatty acid profiles of isolated microorganisms have been widely used in microbial chemotaxonomy, lipid and fatty acid profiles of individual taxa are known to vary quantitatively (and, to a lesser extent, qualitatively) with changes in growth temperature or growth medium (15, 25), leading to skepticism about the reproducibility and interpretation of fatty acid profiles from microbial communities (33). With few exceptions (6, 9, 21, 31, 32), community level fatty acid profiles have been analyzed without any knowledge of the actual taxonomic structure of the community. Although multivariate statistical methods strengthen the ability to discriminate between community level profiles, without knowledge of the underlying taxonomic structure of the data, the biological basis of such discrimination has remained unresolved.

Our objectives were to examine the accuracy and reproducibility of whole-community fatty acid profiles of model soil bacterial communities for which the biomass, taxonomic structure, and potential effects of selected growth environment variables were known a priori and to interpret multivariate statistical analysis of community profiles in the light of this knowledge. By using a commercially available (Microbial ID, Inc. [MIDI], Newark, Del.) gas chromatograph-software system, we obtained whole-cell fatty acid methyl ester (FAME) profiles for 10 soil bacterial isolates under a variety of environmental conditions. We then constructed two model bacterial communities by combining these isolates in various proportions and analyzed the resulting community fatty acid

* Corresponding author. Mailing address: U.S. Geological Survey, Water Resources Division, 6520 Mercantile Way, Suite 5, Lansing, MI 48911-5991.

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profiles by the same methods. Finally, having the advantage of knowing the underlying structure of the data and the ecological significance of profile variation, we evaluated how well and on what basis principal-components analysis (PCA) could differentiate the two communities.

MATERIALS AND METHODS

Bacterial isolates. Ten bacterial cultures were selected arbitrarily from a larger stock of 100 originally isolated from agricultural research plots of the long-term ecological research site at the Kellogg Biological Station, Michigan State University, East Lansing. These isolates were presumed to be representative of natural soil bacteria. All cultures had been isolated on R2A agar (Difco, Detroit, Mich.) and maintained on R2A agar slants in a refrigerator with regular subculturing. For the experiments reported herein, these 10 isolates were routinely cultured at 28°C on R2A agar or in broth medium with the same chemical composition as R2A agar (designated R2A broth). Broth cultures were aerated by shaking. Standard curves correlating cell number (obtained by acridine orange direct counts) and biomass (micrograms of weight per milliliter) with optical density at 590 nm (OD_{590}) were obtained for each isolate during growth in R2A broth at 28°C. These values were used for estimations of cell number or biomass in all experiments.

Fatty acid analysis. Total cellular fatty acids were analyzed by using the MIDI system in accordance with the protocols for cultures grown on solid medium and instrument specifications recommended by the manufacturer. Briefly, our procedures entailed (i) saponification of whole-cell preparations (cells from 20 to 40 ml of a broth culture) at 100°C with 1 ml of methanolic NaOH (15% [wt/vol] NaOH in 50% [vol/vol] methanol), (ii) esterification of the fatty acids at 80°C with 2 ml of 3.25 N HCl in 46% (vol/vol) methanol, (iii) extraction of the FAMEs into 1.25 ml of 1:1 (vol/vol) methyl-*tert*-butyl ether–hexane, (iv) aqueous washing of the organic extract with 3 ml of 1.2% (wt/vol) NaOH, and (v) analysis of the washed extract by gas chromatography.

Reference MIDI-FAME profiles after growth on R2A agar at 28°C were obtained for each of the 10 isolates upon first culture. During the course of our experiments, at least six replicate profiles from growth on R2A agar at 28°C and eight replicate profiles from growth in R2A broth at 28°C were obtained for each isolate. In addition, three replicate profiles were obtained for each of the 10 isolates which grew under the following conditions: (i) on Nutrient agar (Sigma) or tryptic soy broth agar (TSBA; tryptic soy broth solidified with 3% [wt/vol] agar; Sigma) at 28°C and (ii) in R2A broth at 17 or 32°C. Finally, replicate MIDI-FAME profiles were obtained for each isolate in the early logarithmic (EL), mid-logarithmic (ML), early stationary (ES), and, for selected isolates, late stationary (LS; 24 h) stages of growth in R2A broth at 28°C. Culture growth from agar media was typically harvested after 24 to 48 h; the ML growth phase was typically reached by most broth cultures after 12 to 16 h at 28°C.

A comparison of MIDI-FAME whole-cell extract profiles with phospholipid fatty acid (PLFA) profiles and a comparison between MIDI-FAME and PLFA yield on a unit of dry weight basis was made for two selected isolates (*Bacillus* sp. strain 94 and *Cytophaga* sp. strain 72; strain numbers refer to sequence within the original 100 isolates noted above). Cultures grown in R2A broth at 28°C were subsampled at the EL, ML, and ES growth stages, as estimated by OD_{590}, and replicate cell pellets obtained by centrifugation (10,000 × *g*, 20 min) were analyzed by the MIDI-FAME protocol modified to give quantitative recovery of FAME. This modification included two additional extractions with methyl-*tert*-butyl ether–hexane, drying of the combined extracts under N_2, and resuspension of the dried extracts in 500 μl of methyl-*tert*-butyl ether–hexane. Subsamples of these same cultures were kindly analyzed at the Kellogg Biological Station by Søren O. Petersen (University of Aalborg, Aalborg, Denmark) for PLFA by (i) extraction of lipids from culture pellets by a modified Bligh-Dyer (3) procedure (23) utilizing dichloromethane in place of chloroform, (ii) separation of phospholipids by preparative solid-phase extraction (16), and (iii) preparation of phospholipid FAMEs by basic transmethylation (7). FAME extracts obtained by both the MIDI-FAME and PLFA methods were analyzed with the MIDI system. FAME yield was quantified by Søren O. Petersen through calibration of the MIDI gas chromatography response in area units with respect to known amounts of a 19:0 FAME standard (Sigma). Finally, replicate biomass (micrograms of dry weight per milliliter of culture) estimates were obtained for subsamples of the same cultures taken at the same time as the samples used for MIDI-FAME and PLFA analyses.

Fatty acids were designated in the form X:Y, where X is the total number of carbon atoms and Y is the number of double bonds present. If double bonds are present, the location is designated ωZc or t, where Z indicates the number of carbon atoms from the aliphatic (ω) end of the molecule and c or t represent *cis* or *trans* geometry, respectively. In addition, i (*iso* branching), a (*anteiso* branching), and the location of hydroxy (OH), cyclopropane (cyc or cyclo), or methyl (Me) groups may also be noted. MIDI gas chromatography runs were calibrated against a standard mixture of known fatty acids provided by MIDI. Detected sample peaks were named by interpolation of retention time using the equivalent chain length method. Peaks that did not display equivalent chain length values of known fatty acids were left unnamed.

Model communities. Model bacterial communities were constructed by combining known proportions of cells from 6 of the 10 soil bacterial isolates (community I-A [CI-A] and CI-B) or 4 of the 10 isolates (CII-A and CII-B). A and B designate replicate experiments conducted in November 1992 (A) and April 1993 (B). On both occasions, cultures of the individual isolates were grown to the ML-ES phase in R2A broth at 28°C, as estimated by OD_{590}. The OD_{590} was correlated with the cell count (cells per milliliter) or biomass (micrograms [dry weight] per milliliter) from standard curves previously established for each isolate. Culture volumes required to achieve the desired proportions of isolates (see below) were combined immediately before analysis and centrifuged to obtain the community cell pellet, which was resuspended in phosphate buffer (50 mM, pH 7.0). For both experiments A and B, three replicate subsamples of each community were removed from this final resuspension and independently analyzed by the MIDI-FAME protocol described above. CI consisted of equal proportions of the following six soil bacteria: *Corynebacterium* sp. strain 5, *Pseudomonas* sp. strain 14, *Cytophaga* sp. strains 19 and 72, *Arthrobacter* sp. strain 42, and *Bacillus* sp. strain 81. CII consisted of *Bacillus* sp. strain 94 (which made up one-half of the community on a cell number or biomass basis) and *Corynebacterium* sp. strain 5, *Pseudomonas* sp. strain 31, and *Cytophaga* sp. strain 47 (which each made up one-sixth of the community).

Statistical analysis. Means and standard deviations for individual fatty acids in replicate subsample FAME profiles were determined by using SigmaStat (Jandel Scientific, San Rafael, Calif.). PCA was performed by using SAS/STAT,

TABLE 1. Characteristics of the 10 soil bacterial isolates studied

Isolate	Gram reaction	Motility	Pigment[a]	Cell shape	Spores	BIOLOG ID[b] (similarity index)	MIDI ID[c] (similarity index)
Corynebacterium sp. strain 5	+	None	Yellow	Clublike, snapping division	None	*C. fascians* (0.363)	*C. fascians* (0.224)
Pseudomonas sp. strain 14	−	+	White	Small rods	None	*P. cepacia* (0.478)	NG[d]
Pseudomonas sp. strain 31	−	+	White	Moderate rods	None	*P. fluorescens* B (0.639)	*P. syringae* (0.639)
Arthrobacter sp. strain 42	+	None	White	Pleoform rods	None	*None*	*A. globiformis* (0.090)
Arthrobacter sp. strain 53	+	None	Yellowish	Pleoform rods	None	*A. histidinolovorans* (0.026)	*A. histidinolovorans* (0.504)
Cytophaga sp. strain 19	−	+ (gliding)[e]	Pink	Very small rods	None	*Sphingobacterium thalpophilum* (0.452)	*C. heparina* (0.132)
Cytophaga sp. strain 47	−	+ (gliding)	Gold[f]	Long, thin rods	None	*C. johnsonae* (0.218)	NG
Cytophaga sp. strain 72	−	+ (gliding)	Gold[f]	Long, thin rods	None	*C. johnsonae* (0.385)	*C. johnsonae* (0.097)
Bacillus sp. strain 81	+	+	White	Large rods	+ (terminal, round)	*Corynebacterium pseudodiphtherium* (0.294)	*B. sphaericus* (0.818)
Bacillus sp. strain 94	+	None	Cream	Large, rectangular rods	+ (subterminal, oval)	*Corynebacterium pseudodiphthericum* (0.406)	*B. maroccanus* (0.828)

[a] Color of colony after growth on R2A agar.
[b] After 24-h reaction in BIOLOG GN (gram-negative) or GP (gram-positive) plates, as appropriate. Values of <0.5 after 24 h are considered unacceptable identifications by BIOLOG.
[c] After overnight growth on TSBA solid medium. Values of >0.5 are considered a good match.
[d] NG, no growth on TSBA medium.
[e] As observed microscopically after growth in R2A broth medium to the ML phase.
[f] Flexirubin presumed as evidenced by characteristic color change in alkaline or acidic solution (26).

version 6.03 (SAS Institute, Cary, N.C.), by following the PRINCOMP procedure.

RESULTS

Isolate fatty acid profiles. The 10 bacterial isolates were assigned to five bacterial genera on the basis of the characteristics listed in Table 1 plus characteristics of MIDI-FAME profiles of cultures grown to the ML-ES phase in R2A broth at 28°C (the standard growth conditions for our community experiments). Table 2 shows a single, typical MIDI-FAME profile for each isolate after growth under these standard conditions. Comparison of eight separate analyses for each isolate under these standard conditions showed a consistent, reproducible pattern, or reference profile, which exhibited characteristic dominant fatty acids (see below). The coefficient of variation for most fatty acids was generally <10% over the eight separate analyses, even though samples were obtained over a period of months. In addition, fatty acid profiles for isolates grown on R2A agar at 28°C remained very similar to the original profiles recorded (in 1989 or 1990) for each isolate after growth under these same conditions.

MIDI-FAME analysis produced isolate profiles that were characteristic of the assigned genus, as reported in the literature (15, 25, 30), under all of the growth conditions tested. Specifically (Table 2), the *Pseudomonas* isolates exhibited equivalent proportions of 16:0 and 16:1 ω7c, high proportions of the 18:1 ω7c-ω9t-ω12t group, and no branched-chain fatty acids; the *Bacillus* profile was characterized by high proportions of branched-chain fatty acids and the presence of 16:1 ω7c alcohol and 16:1 ω11c; the *Cytophaga* profile was characterized by branched-chain fatty acids combined with a high proportion of straight- and branched-chain 2-OH and 3-OH fatty acids; the *Corynebacterium* profile exhibited high proportions of 18:1 ω9c and 18:0 10 Me; and the *Arthrobacter* profile was distinguished by the presence of extremely high proportions of 15:0a and 17:0a fatty acids. Some fatty acids occurred in only one profile type, e.g., 18:0 10 Me in the *Corynebacterium* sp. strain 5 profile, 18:1 ω7c-ω9t-ω12t in the *Pseudomonas* profile, 15:0i 3-OH or 17:0i 3-OH in the *Cytophaga* profiles, and 16:1 ω7c alcohol in the *Bacillus* profiles. No fatty acid occurred at a high proportion in all isolates. Two isolates did not grown on TSBA medium (recommended by MIDI as the growth medium for taxonomic analyses). Of the remaining eight, only three were identified to the species level with a profile similarity index of >0.5 (range, 0 to 1.0) by MIDI-FAME (Table 1). MIDI specifies similarity indices of >0.5 as having a high probability of being correct and those between 0.6 and 1.0 as being excellent matches.

The 10 isolates used for these studies were a subset of a larger group of 100 soil bacteria that had been selected as representative of the soil community. Of these original 100, 11 had profiles similar to that of *Corynebacterium* sp. strain 5, 12 had profiles similar to those of *Pseudomonas* sp. strains 14 and 31, 15 were similar to the *Bacillus* sp. strain 81 and 94 profiles, 18 were similar to the *Cytophaga* sp. strain 19, 47, and 72 profiles, and 19 were similar to the *Arthrobacter* sp. strain 42 and 53 profiles, accounting for 75 of the 100 original isolates. This suggested that the 10 isolates used in our studies were representative of this larger group.

Environmental effects on MIDI-FAME profiles. To determine the impact of environmental variables on community level FAME profiles, we assessed the effects of temperature, growth medium, plate versus broth culture in the same me-

TABLE 2. FAME profiles of the 10 selected bacterial isolates under typical growth conditions[a]

Fatty acid[b]	% of total fatty acids									
	Coryne-bacterium sp. strain 5	*Pseudo-monas* sp. strain 14	*Pseudo-monas* sp. strain 31	*Arthro-bacter* sp. strain 42	*Arthro-bacter* sp. strain 53	*Cytophaga* sp. strain 19	*Cytophaga* sp. strain 47	*Cytophaga* sp. strain 72	*Bacillus* sp. strain 81	*Bacillus* sp. strain 94
10:0 3-OH			3.91							
12:0	2.41		4.76							
12:0 2-OH			3.39							
12:0 3-OH			2.61							
13:0i							0.38	0.30		
14:0	9.28	4.19	0.60	1.67	0.70	0.98	1.00	1.64		3.14
14:0i				1.01	1.37				2.90	4.15
14:0 2-OH						0.27				
15:0	2.12					0.84	5.32	5.04		
15:0i				2.46	3.22	34.50	26.16	28.15	51.16	7.07
15:0a				72.10	75.98	2.89	4.84	3.44	10.05	68.59
15:0 2-OH						0.44	0.55	0.4		
15:0i 3-OH						3.49	5.78	6.92		
15:0i 2-OH or 16:1 ω7t[c]	12.75		8.86			9.92				
16:0	26.76	19.87	27.22	3.43	1.47	6.18	8.68	17.58	1.07	5.86
16:0i				3.65	4.57	0.57	2.13	0.81	10.05	2.37
16:0 3-OH						2.43	2.16	8.43		
16:0i 3-OH						1.31	1.02	1.12		
16:1 ω7c		25.18	36.60	2.79		9.82	12.96	14.79		
16:1 ω7c alc									11.44	2.06
16:1 ω11c									1.49	4.56
17:0i						0.89	1.53	1.12	4.67	
17:0a				9.81	12.70				2.92	2.20
17:0 2-OH						0.97	0.61	0.50		
17:0i 3-OH						19.67	5.89			
17:0 cyclo			0.80							
Iso-17:1 ω9c						1.95	5.06	1.56		
Ante-17:1 ω9c				2.33						
18:0	2.46	1.73	0.41							
18:1 ω9c	34.75									
18:0 10 Me	7.73									
18:1 ω7c, ω9t, or ω12t[c]		39.97	10.82							

[a] Fatty acid profiles were determined by MIDI-FAME analysis. Cultures grown to the ML phase at 28°C in R2A broth.
[b] The fatty acids listed are those typically found in one or more isolates at >0.5% of the total.
[c] This is a group that cannot be reliably separated by MIDI.

dium, and growth phase on the fatty acid profiles of five isolates representative of the five genera to which our isolates were assigned (Tables 3 to 5). In general, genus-specific profiles remained clearly distinguishable from each other, regardless of modifications to the growth environment. However, each genus-specific profile contained a subset of fatty acids, often comprising a large percentage of the total, for which the relative proportions changed, sometimes dramatically, under different growth conditions. For example, an increase in growth temperature significantly increased the relative proportion of 15:0i in *Bacillus* sp. strain 94 and *Cytophaga* sp. strain 19, with a concomitant loss of 16:1 ω7c alcohol and 16:1 ω7c, respectively (Table 3). This increase in short, branched-chain fatty acids that occurred at high growth temperatures has been previously documented for *Bacillus* spp. (17) but not for *Cytophaga* spp. These fatty acids are believed to influence membrane fluidity in bacterial taxa for which the fatty acid profile is dominated by branched-chain rather than straight-chain fatty acids. As a second example, *Pseudomonas* sp. strain 31 exhibited a loss of the monoenoic fatty acid 16:1 ω7c and a proportional increase in the corresponding cyclo form (17:0 cyc) in the LS growth phase or on any solid medium (Table 4). This change has been previously noted for other *Pseudomonas* spp. in the stationary phase of growth (25). *Corynebacterium* sp. strain 5 exhibited more subtle changes in the relative proportions of 16:0, 18:1 ω8c, and the 15:0i 2-OH–16:1 ω7t group with changes in the growth environment (Table 4). The relative proportion of 18:0 10 Me in this species was more variable, always >5%, and showed no predictable trends. *Arthrobacter* sp. strain 53 exhibited very stable profiles, with 15:0i always >60% and 17:0a generally >9% of the profile regardless of the medium, temperature, or growth phase (Table 5).

We did not observe unique effects of different growth media on isolate fatty acid profiles. Instead, for all of our isolates, MIDI-FAME profiles after 24 to 48 h of growth on solid medium were often similar to profiles of LS phase (24 h) broth cultures (Tables 3 to 5).

Quantitative estimates of FAME yield. To quantify biomass, and especially to determine the abundance of any given organism in a community level FAME profile, the FAME yield must be reproducible and relatively constant over a wide variety of taxa. We estimated MIDI-FAME yield from the relationship between MIDI-FAME area units and culture volumes for broth cultures with known OD_{590}s. One MIDI-FAME area unit was determined to represent 0.73 ng of a 19:0

TABLE 3. Effect of plate culture medium and broth culture temperature and growth phase on FAME profiles of selected isolates

Fatty acids[a]	% of total fatty acid in *Cytophaga* sp. strain 19									% of total fatty acids in *Bacillus* sp. strain 81								
	Medium[b] (plates, 28°C):			Temp, °C (R2A broth):		Growth phase (R2A broth, 28°C):				Medium[b] (plates, 28°C):			Temp, °C (R2A broth):		Growth phase (R2A broth, 28°C):			
	R2A	NA	TSBA	17	32	EL	ML	ES	LS	R2A	NA	TSBA	17	32	EL	ML	ES	LS
14:0	0.62	0.32	0.67	0.75	1.33	1.16	1.07	0.91	0.72									
14:0i										2.83	3.36	5.11	2.91	1.45	2.03	1.53	1.48	2.91
15:0	3.60	2.38	0.29	0.55	0.38	0.43	0.45	0.54	5.23									
15:0i	31.24	32.32	28.34	23.51	49.24	22.41	24.01	24.34	34.50	47.84	56.18	42.55	40.66	57.39	36.30	37.13	36.27	48.34
15:0a	4.62	4.67	1.94	5.77	1.66	1.59	1.56	1.79	3.73	5.68	10.75	9.10	9.38	9.38	27.62	19.96	17.52	7.87
15:0 2-OH	0.96	1.49	0.15	0.81		0.41	0.33	0.37	1.31									
15:0i 3-OH	1.89		1.93	2.97	3.59	2.69	2.58	2.53	2.64									
15:0i 2-OH or 16:1 ω7t[c]	17.18	30.46	17.40	15.17	11.39				17.79									
16:0	3.05		4.06	4.63	10.06	6.90	7.74	6.56	3.20			0.54		1.14	1.93	0.91	1.03	0.52
16:0i	1.09	0.60	0.22	1.87	0.45	0.63	0.61	0.62	0.77	11.90	9.08	15.69	10.82	7.86	6.29	6.93	6.81	10.38
16:0 3-OH		0.62	1.53															
16:0i 3-OH	1.05	1.14	0.55	4.58	0.86	1.21	1.11	1.03	1.16									
16:1 ω5c	2.57	1.90	1.29	3.74	0.52	4.84	3.82	3.26	2.15									
16:1 ω7c	15.20		12.98	21.63	12.09	34.97	32.76	20.15	12.33									
16:1 ω7c alc										18.05	9.58	15.03	20.67	7.15	6.96	10.11	12.43	13.41
16:1 ω11c										2.30	3.89	2.44	1.81	1.48	2.04	2.15	2.21	1.35
17:0i	0.67		1.04	0.54	0.89	0.53	0.55	0.59	0.64	5.36	3.91	3.48	3.31	6.57	4.14	6.06	5.51	5.82
17:0a										1.46	3.25	2.25	2.36	3.70	8.33	7.22	6.57	2.20
17:0 2-OH	0.70	1.19	0.73	3.63	0.34	1.00	1.02	1.09	0.77									
17:0i 3-OH			17.53			15.32	15.60	16.15										
Iso-17:1 ω9c	4.25	10.45	4.48	4.25	1.50	2.82	2.82	3.61	3.52									
18:0															1.82		1.28	0.50

[a] The fatty acids listed are those typically present at >0.5% of the total.
[b] Solid culture media were used, and cells were harvested after 24 to 48 h.
[c] This is a group that MIDI-FAME does not reliably separate.

FAME standard per ml. The estimates of FAME yield for our isolates were as follows (micrograms of MIDI-FAME per microgram of dry weight): *Corynebacterium* sp. strain 5, 0.022 ± 0.007; *Pseudomonas* sp. strain 14, 0.024 ± 0.007; *Pseudomonas* sp. strain 31, 0.032 ± 0.004; *Arthrobacter* sp. strain 42, 0.023 + 0.003; *Arthrobacter* sp. strain 53, 0.027 ± 0.002; *Bacillus* sp. strain 81, 0.020 ± 0.006; *Bacillus* sp. strain 94, 0.011 ± 0.005; *Cytophaga* sp. strain 19, 0.048 ± 0.008; *Cytophaga* sp. strain 47, 0.070 ± 0.006; *Cytophaga* sp. strain 72, 0.041 ± 0.005. MIDI-FAME yield was reproducible for each isolate and varied approximately fivefold across taxa, with consistently higher yields from the *Cytophaga* spp. and the least yield from the *Bacillus* spp. PLFA estimates were made for two isolates representing extremes in MIDI-FAME yield: *Bacillus* sp. strain 94 (0.0031 ± 0.0016 g or 11.4 ± 6.25 μmol of PLFA per g of dry weight) and *Cytophaga* sp. strain 72 (0.0532 ± 0.0245 g or 197 ± 90.7 μmol per g of dry weight). These PLFA yield values fall toward the extremes of values reported in the literature (57 to 354 μmol of PLFA per g of dry weight; 19). Since the MIDI-FAME procedure extracts lipids other than phospholipids (lipopolysaccharide, storage lipids, etc.) MIDI-FAME yields cannot be compared directly with those obtained by the more standard PLFA assay procedures.

Model communities. By combining isolates in various proportions, we constructed two model soil bacterial communities which, when analyzed by MIDI-FAME, would allow us to address questions regarding biomass estimation and fatty acid detection unique to certain isolates within a composite community-level FAME profile. Furthermore, our model communities were constructed on two separate occasions, to allow us to address the reproducibility of community-level MIDI-FAME profiles. Finally, our model community profiles were analyzed by using PCA to address how well, and on what basis, this multivariate statistical method can differentiate profiles of communities with different taxonomic compositions. CI-A, composed of *Corynebacterium* sp. strain 5, *Pseudomonas* sp. strain 14, *Cytophaga* sp. strains 19 and 72, *Arthrobacter* sp. strain 42, and *Bacillus* sp. strain 81 in equal proportions, which was estimated to contain 5×10^8 cells per ml and 213 μg of dry weight per ml, yielded 0.026 ± 0.008 μg of FAME per μg of dry weight from three replicate samples. The MIDI-FAME profile of CI-A is shown in Table 6. The MIDI-FAME profile of CI-B (the identical community constructed in a separate experiment) was very similar to that of CI-A (see below) and yielded 0.022 ± 0.002 μg of FAME per μg of dry weight. For both CI-A and CI-B, FAME profiles were dominated by 15:0i (21.53 ± 0.61 for CI-B), 15:0a (17.13 ± 0.53 for CI-B), 16:0 (11.02 ± 0.38 for CI-B), and 16:1 ω7c (13.60 ± 0.47 for CI-B). In both communities, fatty acids were detected which uniquely indicated the presence of *Bacillus* sp. strain 81 (16:1 ω7c alcohol, 1.26 ± 0.07 in CI-B), *Pseudomonas* sp. strain 14 (17:0 cyclo, 0.69 ± 0.61 in CI-B), *Corynebacterium* sp. strain 5 (18:1 ω9c, 2.04 ± 0.15 and 18:0 10 Me, 0.98 ± 0.11 in CI-B), and both *Cytophaga* spp. (15:0i 3-OH, 3.4 ± 0.10 and *iso*-17:1 ω9c, 2.07 ± 0.07 in CI-B). Although the unique fatty acid for *Arthrobacter* sp. strain 42, *ante*-17:1 ω9c, should have been observed in the community profile, it was not, possibly because of its low abundance in the *Arthrobacter* fatty acid profile (Table 2), combined with the relatively low FAME yield from this isolate (see above). MIDI-FAME profiles for CI were highly reproducible in terms of fatty acid yield and relative abundance of specific fatty acids, but not all members of the community could be detected.

CII-A was composed of *Corynebacterium* sp. strain 5,

TABLE 4. Effect of plate culture medium and broth culture temperature and growth phase on FAME profiles of selected isolates

Fatty acid[a]	% of total fatty acids in *Corynebacterium* sp. strain 5									% of total fatty acids in *Pseudomonas* sp. strain 31								
	Medium[b] (plates, 28°C):			Temp, °C (R2A broth):		Growth phase (R2A broth, 28°C):				Medium[b] (plates, 28°C):			Temp, °C (R2A broth):		Growth phase (R2A broth, 28°C):			
	R2A	NA	TSBA	17	32	EL	ML	ES	LS	R2A	NA	TSBA	17	32	EL	ML	ES	LS
10:0	0.50	NG[c]	0.15		NG			0.34				0.19		NG				
10:0 3-OH										3.21	3.56	4.06	3.75		3.49	3.38	3.28	3.03
12:0	0.43		0.36	0.42		0.79	0.56	0.48	0.43	3.22	2.92	3.21	3.65		3.42	3.64	3.63	3.13
12:0 2-OH										4.65	4.31	3.88	3.21		2.62	2.14	2.27	3.47
12:0 3-OH										3.09	4.94	4.72	1.71		3.14	2.63	3.08	3.51
13:0			1.30															
14:0	9.09		6.19	9.59		11.90	9.53	9.05	9.83	0.48	0.61	0.41	0.39		0.63	0.55	0.45	0.62
15:0	2.25		12.23	2.56			1.16	1.06	1.26		0.31							
15:0i 2-OH or 16:1 ω7t[d]	16.23		14.27	24.24		14.04	12.53	13.49	13.21						12.57	12.33	9.79	8.70
15:1 ω5c			4.43															
16:0	25.55		14.77	18.11		26.89	26.46	27.28	30.25	34.14	33.55	28.92	21.60		31.76	32.16	30.51	31.63
16:1 ω7c	0.90		0.82	1.25		1.90	1.53	1.43	1.24	18.89	23.97	37.27	45.67		29.37	29.56	30.78	28.47
16:1 ω9c			0.28	0.61					0.33									
17:0										0.37	0.46	0.18						
17:0 10 Me	0.39		1.72															
17:0 cyc										19.89	14.36	0.90					0.24	1.45
17:1 ω8c	1.19		6.75	2.09			0.94	1.03	0.95									
Iso-17:1 ω9c			0.34															
18:0	0.48		1.81	0.70		1.16			1.19	1.35	0.96	1.37	0.67		0.44	0.50	0.78	1.27
18:0 10 Me	15.13		6.92	5.66		5.64	7.72	8.05	7.31									
18:1 ω9t-ω12t-ω7c[d]										9.93	9.76	14.25	18.82		12.55	13.12	15.19	14.74
18:1 ω9c	22.69		19.58	28.32		35.59	36.81	34.87	30.30									
19:0	0.80		0.88	0.86				0.71	1.48									
19:1/unknown[d]	3.19		5.48	4.85		2.08	1.92	2.17	2.22									

[a] The fatty acids listed are those typically present at >0.5% of the total.
[b] Solid culture media were used, and cells were harvested after 24 to 48 h.
[c] NG, no growth under this condition.
[d] This is a group that MIDI-FAME does not reliably separate.

Pseudomonas sp. strain 31, *Cytophaga* sp. strain 47, and *Bacillus* sp. strain 94. *Bacillus* sp. strain 94 comprised one-half of the community on a cell number or biomass basis, while the other three isolates each comprised one-sixth of the community. CII-A was estimated to contain 2.4×10^8 cells per ml and 124 μg of dry weight per ml and yielded 0.023 ± 0.002 μg of FAME per μg of dry weight. The MIDI-FAME profile of CII-A is shown in Table 6. CII-B, a replicate community of isolate proportions identical to those of CII-A but constructed in a separate experiment, yielded 0.010 ± 0.002 μg of FAME per μg of dry weight and FAME samples for this community needed to be concentrated. During the concentration procedure, contaminating hydrocarbons >17 carbon atoms long were introduced; hence, only FAMEs of <17:0 were considered in the analysis of CII-B. The same fatty acids as for CII-A dominated the profile of CII-B: 15:0i (9.19 ± 0.095), 15:0a (29.36 ± 0.54), 16:0 (13.09 ± 0.85), and 16:1 ω7c (6.87 ± 0.13). These same fatty acids dominated profiles from CI-A and CI-B, but in CII-A and CII-B they appeared in different proportions. In both CII-A and CII-B, the characteristic 12:0,

TABLE 5. Effect of plate culture medium and broth culture temperature and growth phase on the FAME profile of *Arthrobacter* sp. strain 53

Fatty acid[a]	% of total fatty acids in *Arthrobacter* sp. strain 53								
	Medium[b] (plates, 28°C):			Temp, °C (R2A broth):		Growth phase (R2A broth, 28°C):			
	R2A	NA	TSBA	17	32	EL	ML	ES	LS
14:0	0.66	0.52	0.67			1.36	1.30	0.92	0.68
14:0i	1.71	1.18	1.68	2.16	1.08	1.65	1.52	1.20	2.06
15:0	0.69	0.38	0.20			0.54	0.55	0.46	0.31
15:0i	6.94	8.44	3.13	3.57	4.07	2.47	2.33	2.14	5.39
15:0a	64.55	65.60	76.33	82.44	67.03	76.39	75.34	74.23	76.47
16:0	1.83	1.39	1.69	1.04	2.56	3.25	3.09	2.66	1.03
16:0i	9.01	5.39	6.03	4.56	6.64	5.24	5.66	5.36	5.38
17:0i	0.77	1.07	0.25						0.31
17:0a	13.84	16.02	9.86	4.34	18.61	9.09	10.22	12.68	8.37

[a] The fatty acids listed are those typically present at >0.5% of the total.
[b] Solid culture media were used, and cells were harvested after 24 to 48 h.

TABLE 6. FAME profiles of two model microbial communities composed of soil bacterial isolates

Fatty acids[a]	Mean % of total fatty acids ± SD	
	CI-A[b]	CII-A[b]
10:0 3-OH		0.85 ± 0.13
12:0		1.35 ± 0.03
12:0 2-OH		0.58 ± 0.05
12:0 3-OH		0.67 ± 0.12
13:1	1.63 ± 0.44	0.87 ± 0.23
14:0	3.00 ± 0.26	2.50 ± 0.06
14:0i	0.11 ± 0.20	1.42 ± 0.13
15:0	1.31 ± 0.12	2.22 ± 0.13
15:0i	22.19 ± 1.20	11.31 ± 0.40
15:0 2-OH	0.82 ± 0.13	1.95 ± 0.09
15:0a	10.96 ± 0.11	20.98 ± 0.64
15:0i 3-OH	1.98 ± 0.16	1.45 ± 0.02
15:0i 2-OH or 16:1 ω7t[c]		3.67 ± 0.07
16:0	15.80 ± 0.36	21.17 ± 0.34
16:0i	1.94 ± 0.06	1.51 ± 0.04
16:0 3-OH	0.50 ± 0.87	0.39 ± 0.34
16:0i 3-OH	1.15 ± 0.64	
16:1 ω5c	0.85 ± 0.05	
16:1 ω7c	10.47 ± 0.45	7.75 ± 0.35
16:1 ω7c alc	0.69 ± 0.07	
16:1 ω11c	0.16 ± 0.14	
17:0i	1.43 ± 0.08	1.07 ± 0.26
17:0a	3.55 ± 0.22	1.26 ± 0.15
17:0i 3-OH	2.23 ± 0.38	1.15 ± 0.20
17:0 cyclo	2.83 ± 0.63	1.79 ± 0.15
Iso-17:1 ω9c	1.21 ± 0.04	0.70 ± 0.13
18:0	0.68 ± 0.34	0.94 ± 0.08
18:1 ω9c	3.66 ± 0.46	3.95 ± 0.11
18:0 10 Me	1.68 ± 0.22	1.91 ± 0.38
18:1 ω7c or ω9t or ω12t[c]		3.15 ± 0.12
19:0 cyclo	1.06 ± 0.10	

[a] Fatty acid profiles were obtained by MIDI-FAME analysis (see text).
[b] CI-A and CII-A are described in the text. The data shown are for three replicate subsamples.
[c] This is a group not reliably separated by MIDI-FAME.

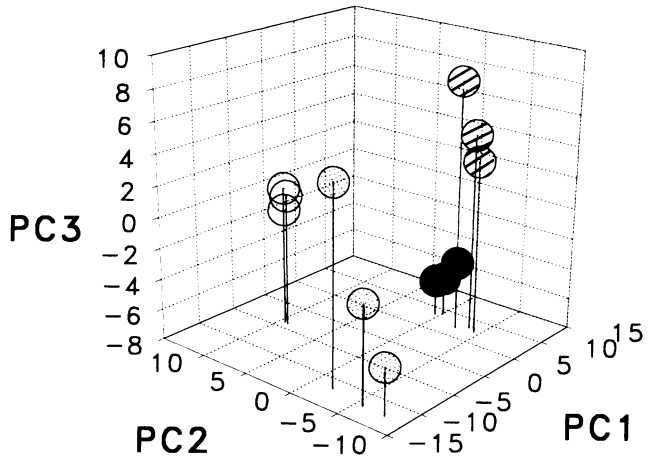

FIG. 1. Three-dimensional plot of the distribution of six replicates of two model bacterial communities (CI and CII) on the first three PCs (PC1, PC2, and PC3) and after PCA of their fatty acid profiles. Symbols: ●, CI-A; ⊘, CI-B; ⊙, CII-A; ●, CII-B.

12:0 2-OH, 12:0 3-OH, and 10:0 3-OH of *Pseudomonas* sp. strain 31 were detected at low levels. Also detected in both CII-A and CII-B were the *Cytophaga* sp. strain 47 fatty acid 15:0i 3-OH (2.19 ± 0.074 in CII-B) and the 14:0i fatty acid of *Bacillus* sp. strain 94 (3.65 ± 0.070 in CII-B). The 18:1 ω9c and 18:0 10 Me fatty acids of *Corynebacterium* sp. strain 5 were detected in CII-A (Table 6) but not in CII-B because of the presence of contaminating peaks at similar retention times. MIDI-FAME profiles for CII were not as reproducible in terms of fatty acid yield as were the profiles of CI; however, the relative abundances of specific fatty acids were similar in the two CII profiles, and each member of the community could be detected.

PCA. In addition to determining how well each community level MIDI-FAME profile represented the community's biomass and taxonomic structure, we also wished to know if the two community level profiles could be distinguished from each other by using multivariate statistics. We chose to use PCA, which has been recently used in analyses of community level fatty acid profiles of environmental samples (1, 14, 27). PCA was conducted by using values for each fatty acid (percentage of total fatty acids) which appeared in any of the three replicates of CI or CII, a total of 37 fatty acids. Six of these fatty acids are not presented in Table 6 since they occurred at low levels (<0.3%) or were absent from some replicates. PCA scores obtained after analysis of the covariance matrix are depicted in Fig. 1 in three dimensions corresponding to the first three principal components (PCs). This figure indicates clear separation of CI-A and CI-B replicates from CII-A and CII-B replicates on the first PC. There was close clustering of CI-A and CI-B replicates and weaker clustering of CII-A and CII-B replicates, probably because of the necessity of dropping all fatty acids of >17:0 from the CII-B profile (see above). The first PC accounted for 57.1%, the second PC accounted for 24.9%, and the third PC accounted for 14.6% of the variation in the data, for a cumulative total of 96.6%. PC 1 had high loadings on 15:0i (0.791), 16:1 ω7c (0.364), 17:0a (0.211), and 15:0a (−0.273). PC 2 had the highest loading on 16:0 (0.847), and PC 3 had the highest loading on 15:0a (0.814).

DISCUSSION

In this study, we compared whole-community fatty acid profiles by using PCA and examined how accurately and reproducibly community profiles reflected the underlying taxonomic structure of communities with known compositions. Fatty acid analyses conducted by other researchers have purported to provide estimates of microbial biomass and offer insights into the taxonomic structure of a variety of microbial communities. Our results suggest that this approach has great potential as a routine analytical procedure for assessing whether microbial communities are similar or not. When combined with other measures of community structure and function, this information can be very valuable. However, our results also suggest that interpretation of community fatty acid profiles in terms of biomass and taxonomic composition must be viewed with caution.

There has been much emphasis on the necessity of confirming fatty acid peak identification obtained by gas chromatography (as in the MIDI system) with alternative approaches, such as mass spectrometry (13, 34). This has undoubtedly led to the concern that fatty acid profile analysis is a labor- and cost-intensive approach best left to specialists. Although confirmation is certainly required in those cases in which unique fatty acids and new molecular structures must be analyzed for

taxonomic purposes, our results suggest that fatty acid profiles obtained with the commercially available MIDI-FAME system provide information equivalent to that obtained by more traditional, and lengthier, approaches to PLFA analyses. We found the MIDI-FAME whole-cell fatty acid profiles of our isolates to be highly reproducible under specified growth conditions and similar to PLFA profiles reported in the literature for each of the five genera represented by our isolates. Furthermore, analysis of MIDI-FAME profiles of combinations of our isolates resulted in no spurious or questionable peaks; each fatty acid named was associated with one or more of the isolates in the combination. The MIDI-FAME extraction protocol is different from that typically employed in PLFA analyses, particularly in that it employs a highly alkaline step. The appropriateness of the extraction procedure should be evaluated for any proposed application.

Taxonomic information from fatty acid profiles. Unfortunately, even the most accurate peak identification technology offers no help in overcoming the difficulty of assigning taxonomic interpretations to community level fatty acid profiles. One impediment to taxonomic interpretation is the lack of easily accessed information on the qualitative and quantitative distribution of fatty acids across microbial taxa and within-taxon responses to changes in the growth environment. No database exists which correlates specific fatty acids with the taxa in which they occur, and valuable compendia of microbial lipids, such as that of Ratledge and Wilkinson (25), are indexed not by fatty acids but by taxa. Generalizations about the distribution of certain fatty acids across microbial taxa and about the relative amounts of fatty acids in microbial cells have occurred in the literature dealing with the analysis of whole-community fatty acid profiles (see below). Our results suggest that some of the published biomass estimates and conclusions about community taxonomic composition derived from analysis of community level fatty acid profiles have been based on inappropriate generalizations and that taxonomic interpretations is hindered by significant variation in the presence and yield of "signature" fatty acids across taxa.

For example, it is widely stated that branched-chain fatty acids are characteristic of gram-positive bacteria (15, 25), and while this statement is true, it is not exclusive. Our data (Table 2), as well as those of others (17, 25, 32), demonstrate that branched-chain fatty acids are also characteristic of the gram-negative genus *Cytophaga* and related bacteria in the genus *Flavobacterium*. Because these bacteria have not been well studied and are not considered to compose a significant part of soil, sediment, or epilithic microbial communities, there is the danger of examining fatty acid profiles similar to those of our model communities and concluding that the large proportion of 15:0i fatty acid is due solely to the presence of gram-positive genera. This assumption was made by Findlay et al. (8) for marine sediments and Bååth et al. (1) for soils. In another example, the 17:0 and 19:0 cyclo fatty acids have been stated to be associated with anaerobic bacteria (34). This statement is true to the extent that cyclo fatty acids are characteristic of *Clostridium* spp. (25), some sulfate-reducing bacteria (6, 29), *Vibrio cholerae* (13), and some members of the family *Enterobacteriaceae* under some physiological conditions (11). However, these fatty acids are not exclusive to these groups and occur quite widely in many bacterial genera (25), including the *Pseudomonas* species in our model communities, and were noted in the community level fatty acid profiles of rhizosphere bacterial communities (31, 32). Nevertheless, Scholz and Boon (27) concluded that cyclo fatty acids in community level fatty acid profiles from stream biofilms are indicative of anaerobic bacteria.

Biomass estimates from fatty acid profiles. The use of fatty acid analysis for the quantification of microbial biomass is complicated by the presence of different lipid amounts in different microorganisms. Many studies have used the widely reported conversion factor of 100 μmol of PLFA per g of dry weight of microbial cells (e.g., references 2, 19, 20, 24, 27, and 29). However, specific determinations of the PLFA yield for some organisms have been found to vary widely: *Methylococcus* sp., 272 to 354 μmol of PLFA per g of dry weight; *Methylobacterium* sp., 57 to 66 μmol of PLFA per g of dry weight (19); *Flavobacterium balustinum* 299, 42.3 μmol of PLFA per g of dry weight (32). Our experiments confirm that regardless of whether they are analyzed by MIDI-FAME or PLFA procedures, specific taxa of bacteria may yield significantly different fatty acid amounts (11 to 197 μmol of PLFA per g of dry weight for two representative isolates). Particular problems arise when fatty acids are employed to estimate the biomass of specific groups of organisms in complex communities. Not only is it difficult to convert fatty acid yield into microbial biomass appropriately for individual genera, but inherent taxonomic variation in yield affects whether signature fatty acids are seen at all in community level profiles. For example, the monoenoic fatty acids, such as 16:1 ω7c/t, are widely recognized as being associated primarily with prokaryotes (15, 25) and have been used to draw conclusions about prokaryotic abundance and metabolic status in several environments (1, 8, 14, 19, 24, 27, 29). However, three of the isolates we studied did not contain either isomer of this fatty acid and one exhibited only low levels of 16:1 ω7c (Tables 2 to 5). In our two model communities, 16:1 ω7c (and possibly also 16:1 ω7t, which cannot be distinguished from 15:0i 2-OH by MIDI-FAME) occurred in significantly different proportions and contributed to separation of the communities by PCA (see below). This difference is due primarily to their different taxonomic compositions and not to a difference in prokaryotic abundance or metabolic status. In another example, in CI-A, the presence of *Bacillus* sp. strain 81 is indicated by the 16:1 ω7c alcohol, which was typically found in its fatty acid profile at relatively high abundance (Table 2). However, in CI-A, 16:1 ω7c alcohol was just barely detected (Table 6), in part because of the relatively low yield of fatty acid from this microorganism. We might conclude that *Bacillus* sp. strain 81 was an insignificant component of CI-A, when indeed it was present at a level equal to that of any other member. More dramatically, 16:1 ω7c alcohol was not detected at all in CII-A (Table 6), even though *Bacillus* sp. strain 94, which also exhibits this signature fatty acid (Table 2), made up fully one-half of this community.

Some recent studies have simply recorded the amount of lipid determined and correlated this value with other measurements of microbial biomass (14, 35). Community PLFA yield has been shown to correlate reasonably well with other estimates of microbial biomass in a variety of environments (2, 33, 35). In our experiments, CII-A comprised only one-half of the number of organisms and one-half of the biomass of model CI-A and had a different taxonomic organization; however, while less fatty acid was extracted from CII-A than from CI-A on a per-milliliter basis, the MIDI-FAME yields from both communities were equivalent when converted to a dry-weight basis (CI-A; 0.026 ± 0.008; CII-A, 0.023 ± 0.002 μg of FAME per μg of dry weight). Since MIDI-FAME fatty acids may not be derived solely from phospholipids and since we studied only laboratory cultures, our results do not address whether MIDI-FAME yields would similarly represent biomass in real environments, where starved cells might contain different quanti-

ties of storage lipids or exhibit other physiological adaptations affecting whole-cell fatty acid profiles.

Environmental effects on fatty acid profiles. Our findings on the effects of temperature and growth medium on the fatty acid profiles of specific taxa concur with those reported in the literature (15, 25) in that, within the typical range of conditions over which many bacterial genera grow, there may be significant quantitative changes in the proportions of certain fatty acids (Tables 3 to 5). The most dramatic changes were exhibited by *Cytophaga* sp. strain 19 and *Pseudomonas* sp. strain 31, in which fatty acids present at high proportions under some growth conditions completely disappeared under other conditions (e.g., 15:0i 2-OH–16:1 ω7t in *Cytophaga* sp. strain 19 and *Pseudomonas* sp. strain 31 or 17:0 cyclo in *Pseudomonas* sp. strain 31; Tables 3 and 4). Less dramatic changes which did not involve the complete disappearance of fatty acids also occurred in *Bacillus* sp. strain 81, *Arthrobacter* sp. strain 53, and *Corynebacterium* sp. strain 5 (Tables 3 to 5). For each genus represented in Tables 3 to 5, changes in fatty acids with changes in the growth environment represented a continuum rather than discrete patterns clearly associated with specific environmental variables. For example, the R2A pattern for *Cytophaga* sp. strain 19 was not remarkably different from the LS or the 17°C pattern and the TSBA pattern appeared to be intermediate between the ES and LS patterns. Common features of the profiles shown in Tables 3 to 5 possibly reflect the effects of environmental variables we did not specifically test (e.g., pH and O_2 tension), which could have varied under our test conditions and which are known to affect bacterial fatty acid profiles (25), rather than specific effects of culture medium or age. Significantly, profiles remained characteristic of each genus, regardless of growth condition, since for all isolates, changes occurred in the proportion of only certain fatty acids, while others, often unique to the genus, remained at relatively constant levels. Changes in fatty acid profiles and lipid abundance with changes in the environment have been used to interpret the metabolic status of the community (14, 34). As more is learned about microbial fatty acids, variation with environmental conditions may be used to advantage in ecological interpretations of whole-community fatty acid profiles.

Quantitative changes will affect our ability to interpret more complex fatty acid profiles form whole communities, but it remains to be tested whether such changes would be sufficient to alter a community profile so completely that one might conclude that the community structure had changed. If we constructed CI after growing isolates on agar media, we would expect the CI profile to be different from that shown in Table 6 in that the 15:0i 2-OH–16:1 ω7t group, characteristic of the profile of *Cytophaga* sp. strain 19 after growth on solid media, would be present. In addition, *Pseudomonas* sp. strain 14 often exhibited high proportions of 19:0 cyclo fatty acid after growth on solid media (data not shown), similar to the appearance of 17:0 cyclo in *Pseudomonas* sp. strain 31 (Table 4), and we might therefore expect to see higher proportions of this fatty acid. However, while both 15:0i 2-OH–16:1 ω7t and 19:0 cyclo varied significantly in comparisons of CI with CII, neither was important in distinguishing the two model communities from each other by PCA (see below). In this example, it is likely that the overall community level profile characteristics would remain sufficiently the same to group the agar culture community with the broth culture community by multivariate methods. Our experiments do not allow us to assess whether, for example, the profiles of a soil community at 4°C (winter) and 30°C (summer) would be significantly different even without a change in community taxonomic composition. Seasonal changes in community fatty acid profiles have been noted for lake waters (9) and stream biofilms (27).

PCA. Our results suggest that MIDI-FAME whole-community profiles may be especially valuable as indicators of the degree to which communities are similar or different. Regardless of difficulties associated with determinations of biomass or taxonomic composition, PCA of MIDI-FAME profiles resulted in clear and reproducible separation of the two different model communities and clustering of replicates of each community from both experiments on the first PC axis (Fig. 1). This axis accounted for a significant proportion (57.1%) of the variation in the data, and the first three PCs accounted for virtually all of the variation (96.6%). The first PC had high loadings on 15:0i (0.791), 16:1 ω7c (0.364), and 15:0a (−0.273). These results are obvious after examination of Table 6, which clearly indicates that CI-A and CI-B cluster on the positive end of PC 1 because they exhibit significantly greater amounts of 15:0i and 16:1 ω7c and significantly lesser amounts of 15:0a than do communities CII-A and CII-B. CI-A and I-B are closely clustered on PC 2, but CII-A and CII-B are not, because of the necessity of dropping all fatty acids of >17:0 from the CII-B profile. Nevertheless, CII-B clearly grouped closer to CII-A than to either CI-A or CI-B (Fig. 1). The fact that CII-B was grouped with CII-A even when several fatty acids were deleted from the CII-B profile demonstrates the robust nature of PCA and the general strength of this approach to the comparison of whole-community fatty acid profiles. PC 2 exhibited the highest loading on 16:0, and CII-A and CII-B varied more in this fatty acid than did CI-A and CI-B. Our results are somewhat obvious from examination of Table 6 but would not be so obvious in a comparison of many complex fatty acid profiles with subtle variations, such as those obtained from environmental samples. In such a case, use of PCA would direct attention to those fatty acids which account for significant variation in the data set and might suggest testable hypotheses for further examination.

It is important to note that we used the covariance matrix for PCA. The covariance matrix has favored mathematical properties and is typically used when all of the data are presented in the same units or scales, as in the case for fatty acid profiles reported as percentages, such as ours (5, 10). An alternative approach would be to use the correlation matrix, which has the affect of standardizing the data (5). We also conducted a PCA with the same data but using the correlation matrix (data not presented). In this case, PC 1 accounted for 41.4% of the variation in the data and was equivalently loaded at low levels (~0.24) on several fatty acids (e.g., 16:1 ω5c and 14:0i, among others) which were present in one community but not consistently present in the other. In other words, standardization of this data set emphasized presence or absence over relative abundance.

In this context, it is important to recognize that the amount of lipid extracted from a sample significantly affects the relative amounts of fatty acids reported as percentages of the total, as for our data. Ideally, similar amounts of fatty acids should be compared when using PCA, as well as for taxonomic interpretation. This is in direct contrast to the use of fatty acid profiles for biomass determinations, in which it is important to know whether the lipid amounts are different in different environments.

Conclusions. Data acquired by MIDI-FAME analysis appear to be equivalent to those acquired by the more complex PLFA protocols. MIDI-FAME fatty acid profiles are highly reproducible when obtained from isolates or communities under the same environmental conditions. Factors that influence the accuracy of biomass estimations or taxonomic inter-

pretations from community fatty acid profiles are the same for MIDI-FAME and PLFA analyses. These include (i) the similarity of dominant fatty acids in the profiles of widely different taxa, (ii) lack of unique signature fatty acids as a high percentage of the total in the profiles of many taxa, (iii) taxonomic variation in fatty acid yields, (iv) the relatively immature state of our present knowledge of the quantity and relative distribution of fatty acids across broad taxonomic groups and with respect to environmental variables, and (v) the difficulty of accessing fatty acid data from the literature. Biomass estimations and taxonomic interpretations based on community fatty acid profiles should be approached with caution and supported with data obtained by alternative methods. Nevertheless, whole-community MIDI-FAME profiles indicate the degree to which communities are similar or different and multivariate statistical analyses can be used to differentiate between communities with different taxonomic compositions.

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