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Dynamics of a Microbial Community Associated with Manure Hot Spots as Revealed by Phospholipid Fatty Acid Analyses

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Microbial community dynamics associated with manure hot spots were studied by using a model system consisting of a gel-stabilized mixture of soil and manure, placed between layers of soil, during a 3-week incubation period. The microbial biomass, measured as the total amount of phospholipid fatty acids (PLFA), had doubled within a 2-mm distance from the soil-manure interface after 3 days. Principal-component analyses demonstrated that this increase was accompanied by reproducible changes in the composition of PLFA, indicating changes in the microbial community structure. The effect of the manure was strongest in the 2-mm-thick soil layer closest to the interface, in which the PLFA composition was statistically significantly different ($P < 0.05$) from that of the unaffected soil layers throughout the incubation period. An effect was also observed in the soil layer 2 to 4 mm from the interface. The changes in microbial biomass and community structure were mainly attributed to the diffusion of dissolved organic carbon from the manure. During the initial period of microbial growth, PLFA, which were already more abundant in the manure than in the soil, increased in the manure core and in the 2-mm soil layer closest to the interface. After day 3, the PLFA composition of these layers gradually became more similar to that of the soil. The dynamics of individual PLFA suggested that both taxonomic and physiological changes occurred during growth. Examples of the latter were decreases in the ratios of 16:1ω7*t* to 16:1ω7*c* and of cyclopropyl fatty acids to their respective precursors, indicating a more active bacterial community. An inverse relationship between bacterial PLFA and the eucaryotic 20:4 PLFA (arachidonic acid) suggested that grazing was important.

The addition of cattle manure to soil results in a rapid increase in microbial biomass and activity (1, 19, 23). However, the heterogeneity of the soil-manure system may strongly influence the microbial turnover of substrates like ammonia and easily degradable organic compounds which are normally present in manure at high concentrations. When it is applied to agricultural fields, the distribution of manure is not completely even. Instead, zones in which the soil pores may be saturated with manure are created. From here on, such volumes, where the intensity of microbial activity can lead to partly anaerobic conditions, are referred to as manure hot spots.

Several microbial processes proceed concomitantly within and at short distances from manure hot spots. However, their generally small sizes and irregular shapes make soil sampling and measurements around individual hot spots difficult. Petersen et al. (17, 18) developed a two-phase model system consisting of a manure core surrounded by soil. The well-defined soil-manure interface of this system made it possible to measure concentrations of dissolved organic carbon (DOC), NH_4^+, and NO_3^- on a millimeter scale in and around the hot spot (20). By using microelectrodes, oxygen profiles which showed the dynamics of oxygen consumption during a 3-week incubation period were obtained. Due to diffusional limitations on the flux of oxygen into hot spots and on the fluxes of C- and N-containing compounds out of them, microbial activity became concentrated around the soil-manure interface.

The different carbon and nitrogen transformations that take place in and around a manure hot spot are likely to be reflected in an altered composition of the microbial community. By analyzing the phospholipid fatty acids (PLFA) extracted from the soil, changes in the overall composition of the microbial community can be detected, since different groups of organisms contain different PLFA in their membranes (5, 6, 25). Animal manure itself contains a large number of microorganisms that originate from the gut microflora. For example, Spoelstra (24) counted 10^{10} to 10^{11} cells g (fresh weight)$^{-1}$ in pig slurry, of which 10 to 25% grew on selected media. In the present study, we analyzed the PLFA patterns within and at different distances from manure hot spots, addressing the following specific questions. (i) What are the temporal dynamics of the microbial community? (ii) At what distance from the hot spot can effects on the PLFA pattern, and thus on the community composition, be detected? We also quantified the microbial biomass both as the amount of phosphate liberated from lipid extracts after digestion (lipid-P) and as the total amount of PLFA.

MATERIALS AND METHODS

Soil description, preparation of manure hot spots, and sampling of core layers. The soil used was a Typic Haplumbrept (85% sand, 9.5% silt, 5.5% clay; with 1.21% C and 0.12% N), collected in a fallow field 1 month after a wheat crop had been harvested. After sieving (2-mm-diameter mesh size), the soil was stored at 15°C for 2 weeks. Four days before the start of the experiment, the water content was adjusted to field capacity and the soil was packed to a bulk density of 1.3 g cm^{-3} in cores of polyacrylamide (inner diameter, 44 mm; height of soil core, 70 mm). A number of these cores were used as control samples; the rest were used for preparing manure hot spots.

Manure hot spots were prepared as described by Petersen et al. (20), although only fresh, nondigested manure was used in the experiment. The manure, which

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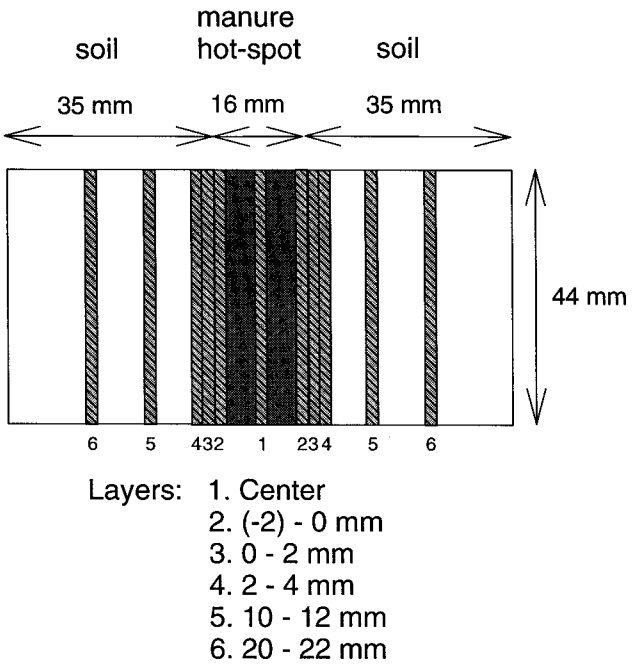

FIG. 1. Two-phase model system with a gel-stabilized mixture of soil and liquid manure placed between two prepacked soil cores. Layers 1 through 6 were used in experiments.

was sieved (1-mm-diameter mesh size) before use, contained 1,087 μmol of DOC and 150 μmol of NH_4^+ g (fresh weight)$^{-1}$. Briefly, manure hot spots (inner diameter, 44 mm; height, 16 mm) were prepared from a mixture (2.5:1:1 [vol/vol/vol]) of soil, manure, and 3% silica gel (18). The mixture contained 180 μmol of DOC g^{-1} and 24.8 μmol of NH_4^+ g^{-1}. The soil cores were sectioned into two parts, with the hot spots placed in the middle (Fig. 1).

Soil cores containing manure hot spots, as well as those without manure, were sealed with Parafilm and incubated at 15°C for 1, 3, 7, 14, or 21 days. Cores were carefully sectioned, and six distance intervals (Fig. 1) were analyzed with regard to PLFA profiles and lipid-P contents (see below). Tubes were incubated vertically. Corresponding layers above and below the central layer were pooled since no gravitational flow of water occurred (data not shown). The concentrations of DOC and inorganic nitrogen, pH, oxygen profiles, and denitrification rates were reported by Petersen et al. (20).

Lipid extraction. Lipids were extracted by the method of Petersen and Klug (16). Samples from layers 1 through 4 (Fig. 1) were obtained from three cores on each sampling occasion (although there were only two samples from layer 1 on day 1 since one sample was lost during preparation); only two replicates were used for layer 5, and one or two replicates were used for layer 6 and control soil. Duplicate samples of manure and soil were taken on day 0. One gram (wet weight) (fresh manure and layers 1 and 2) or 2 g (wet weight) (layers 3 through 6 and control soil) was extracted in 9.5 ml of a one-phase mixture of dichloromethane (DCM), methanol, and K_2HPO_4 buffer (50 mM; pH 7.4) (1:2:0.8 [vol/vol/vol]) for at least 2 h. Thereafter, 2.5 ml of DCM and 10 ml of NaBr (0.8 g ml^{-1}) were added and the phases were allowed to separate. Two 1-ml portions were then taken from the lipid-containing upper phase of each sample for analyses of lipid-P content and PLFA composition. Lipid extracts were dried under a stream of nitrogen and stored at −20°C until analyzed.

The dried lipid extract was dissolved in a small amount of chloroform and fractionated on prepacked columns containing silicic acid (Varian, Harbor City, Calif.). Neutral lipids were eluted with DCM, glycolipids were eluted with acetone, and phospholipids were eluted with methanol. Only the last fraction was retained. Phospholipids were transesterified by a mild alkaline methanolysis, and the resulting fatty acid methyl esters were separated on a capillary gas chromatograph (GC). The GC conditions and procedures for identifying PLFA were those of Frostegård et al. (6).

Fatty acid nomenclature. Fatty acids are designated in terms of the total number of carbon atoms:number of double bonds followed by the position of the double bond from the methyl end of the molecule. *cis* and *trans* configurations are indicated by *c* and *t*, respectively. The prefixes a and i indicate anteiso- and isobranching, respectively; br indicates an unknown methyl branching position; 10Me indicates a methyl group on the 10th carbon atom from the carboxyl end of the molecule; and cy refers to cyclopropane fatty acids.

Determination of microbial biomass. The amount of microbial biomass was determined both as the total amount of PLFA and as the amount of phosphate liberated from the lipid extracts after digestion (lipid-P). For lipid-P determinations, the dried lipid extract was digested in K_2SO_8 at 100°C overnight (17), after which the amount of lipid-P was determined by the method of Van Veldhoven and Mannaerts (26). The amount of bacterial biomass (bacterial PLFA) was estimated from the summed amount of the following PLFA: i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7*t*, i17:0, a17:0, cy17:0, 17:0, 18:1ω7, and cy19:0 (7).

Statistical analyses. The PLFA compositions (individual PLFA expressed in moles percent) of different samples were subjected to principal-component (PC) analyses (PCA). To compare how different pretreatments of data influenced the results of PCA, these analyses were performed in two ways, with either $\log_{10}$-transformed values or a transformation in which the algorithm $x_i \rightarrow \log[x_i/g(x)]$ was calculated (21). In the latter transformation, $i = (1, 2, \ldots n)$ and $g(x)$ is the geometric mean of n values summing to 100%. This algorithm is sometimes needed to avoid so-called closure effects. The meaning of closure effects is that data which sum to a constant, in this case 100%, are constrained in that if one peak increases very much, the percentages of other peaks must decrease. This can be a problem especially when small sets of variables are used (21). Here no such effects were found. The results of PCA by the two methods for data pretreatment were almost identical (the percentages of total variation explained by the first two PCs were 49.4 and 16.5% with $\log_{10}$-transformed data and 48.0 and 17.4% with data transformed by the algorithm); therefore, only data from the simple $\log_{10}$ transformation are presented here. We also analyzed the data by using absolute amounts of the respective PLFA, i.e., in picomoles gram (dry weight [dw]) of soil^{-1}. The PCA plots obtained (not shown) were very similar to the ones obtained by using moles percent. To determine the distance from the soil-manure interface at which a change in the PLFA pattern could be considered statistically significant, a SIMCA analysis was performed (27). A PC model with cross-validation (28) was created for layers 5 and 6; all other layers were then tested for similarity with the model by using $P < 0.05$ as the rejection criterion. The individual PLFA contents in the soil and manure phases (layers 6 and 1, respectively) were analyzed with Student's *t* test.

RESULTS

PLFA compositions and lipid-P contents in soil and manure. Fresh manure contained 316 nmol of lipid-P and 300 nmol of total PLFA g (dw)$^{-1}$ before being mixed with silica gel and soil, while the soil contained 40 nmol of lipid-P and 25 nmol of total PLFA g (dw)$^{-1}$. In the experimental setup, the center of the manure phase (layer 1), which was a 2.5:1:1 mixture of soil, manure, and gel, contained 123 nmol of lipid-P and 71 nmol of total PLFA g (dw)$^{-1}$. The soil and manure differed strongly in PLFA composition. Of the 32 PLFA detected and identified in the soil, 8 were close to or below the detection limit (<50 pmol) in the manure, where a few PLFA dominated. The most abundant PLFA in the manure were palmitic acid (16:0), iso- and anteisobranched i15:0 and a15:0, and monounsaturated 18:1ω7 (Table 1). The proportions, measured in moles percent, of the PLFA i14:0, a15:0, 15:0, a17:0, and 18:1 in the manure phase were between 2 and 3 times higher than those in the soil. Methyl-branched PLFA 10Me16:0, 10Me17:0, and 10Me18:0; branched PLFA br16:0, br17:0, br18:0, and i16:1; monounsaturated 16:1ω5; and cyclopropyl fatty acids cy17:0 and cy19:0 were all found in smaller amounts in the manure phase than in the soil phase.

Temporal dynamics of total PLFA and lipid-P. There was a strong growth of microorganisms around the soil-manure interface, i.e., in layers 2 and 3, during the first days of incubation (Fig. 2). Between days 1 and 3, total PLFA increased from 84 to 132 nmol of PLFA g (dw)$^{-1}$ in layer 2 (−2 to 0 mm) and from 42 to 79 nmol of PLFA g (dw)$^{-1}$ in layer 3 (0 to 2 mm). After this initial period of growth, the microbial biomass gradually decreased in these two layers. Total PLFA remained approximately constant throughout the experiment in soil layers 4 to 6. In the center of the manure core (layer 1), total PLFA decreased from 71 nmol of PLFA (dw)$^{-1}$ at the beginning of the experiment to 50 nmol of PLFA g (dw)$^{-1}$ after 21 days of incubation.

The dynamics of the microbial biomass in different layers showed a similar pattern when the measurements used were

TABLE 1. Amounts of PLFA in the soil (layer 6)[a] and in the manure phase (layer 1)[b]

PLFA	Mean amt of PLFA (mol%)[c]	
	Soil	Manure phase
i14:0[d]	0.75 (0.06)	1.45 (0.07)
14:0[d]	1.83 (0.08)	2.38 (0.02)
i15:0	7.93 (0.18)	7.51 (0.07)
a15:0[d]	5.67 (0.14)	11.8 (0.09)
15:0[d]	0.67 (0.06)	2.06 (0.09)
br16:0[d]	0.48 (0.03)	0.20 (0.02)
i16:1[d]	0.61 (0.04)	0.15 (0.03)
i16:0[d]	2.12 (0.05)	3.06 (0.05)
16:1ω9[d]	1.98 (0.20)	0.97 (0.18)
16:1ω7*c*[d]	8.40 (0.16)	6.25 (0.24)
16:1ω7*t*[d]	0.64 (0.02)	0.76 (0.03)
16:1ω5[d]	4.33 (0.08)	2.13 (0.09)
16:0	18.8 (0.42)	19.4 (0.52)
br17:0[d]	0.36 (0.03)	0.20 (0.02)
10Me16:0[d]	6.55 (0.41)	3.77 (0.09)
i17:0	1.95 (0.06)	1.98 (0.08)
a17:0[d]	0.69 (0.05)	2.17 (0.07)
17:1ω8	0.69 (0.04)	0.73 (0.04)
cy17:0[d]	4.14 (0.08)	2.16 (0.05)
17:0[d]	0.61 (0.04)	1.11 (0.01)
br18:0[d]	0.47 (0.01)	0.26 (0.04)
10Me17:0[d]	0.76 (0.07)	0.38 (0.02)
18:2ω6,9[d]	1.91 (0.13)	2.60 (0.23)
18:1ω9	4.76 (0.18)	5.27 (0.26)
18:1ω7	9.27 (0.17)	9.37 (0.16)
18:1[d]	0.86 (0.04)	2.33 (0.31)
18:0	4.03 (0.41)	3.95 (0.37)
19:1a	0.92 (0.05)	1.13 (0.69)
10Me18:0[d]	1.55 (0.04)	1.15 (0.08)
cy19:0[d]	4.23 (0.17)	2.31 (0.04)
20:4[d]	0.72 (0.06)	0.20 (0.02)
20:0[d]	1.26 (0.10)	0.77 (0.03)

[a] Samples ($n = 7$) from all sampling occasions were used in calculations since no changes in PLFA composition were observed in this layer during the 3-week incubation period.
[b] Samples ($n = 2$) from day 1 were used in calculations.
[c] Data in parentheses are standard errors.
[d] Differences between data from layers 1 and 6 are significant ($P < 0.05$).

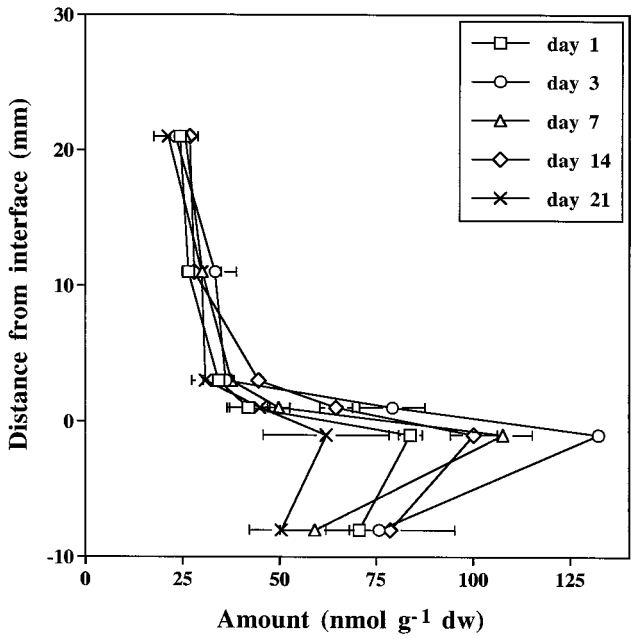

FIG. 2. Temporal dynamics of the microbial biomass, measured as the total amount of PLFA, in different layers. Bars indicate standard errors; $n = 3$ (layers 1 through 4) or 2 (layers 5 and 6).

the amounts of phosphate liberated from phospholipids after digestion (lipid-P) (not shown). Analyses based on combined data from layers 3 to 6 revealed a good correlation ($r = 0.89$; $n = 43$) between total PLFA and lipid-P (Fig. 3). However, the molar ratio, 1:2, between lipid-P and total PLFA predicted by theory was not found. Furthermore, the values for lipid-P in the manure phase (layers 1 and 2) appeared to be consistently higher than the corresponding values for total PLFA.

Temporal dynamics of the PLFA composition. The plot of PC scores (Fig. 4a) illustrates the variations in PLFA profiles of different layers within and outside the manure core during the 21-day incubation period. The first PC, which accounted for 49.4% of the total variation, separated the manure phase from the soil phase, while the second PC (16.5% of the variation) separated the samples over time. Samples from the manure phase were found on the left in the plot, whereas samples from soil layers 4 to 6 were found on the right. The samples from layer 3, just outside the interface (0 to 2 mm), were separated both from the samples taken from the manure phase and from those taken from the soil phase. SIMCA analysis revealed that the PLFA pattern in this layer was statistically significantly different ($P < 0.05$) from that in layers 5 and 6 and thus was influenced by the manure throughout the incubation period. A small effect of the manure was also indicated for samples from layer 4 (2 to 4 mm), which were slightly separated from samples from layers 5 and 6 in the PC plot, although this was not statistically significant for all sampling occasions (SIMCA analysis). Control cores containing only soil had a PLFA pattern almost identical to that of soil layer 6 in manure-containing cores. Hereafter, layer 6 is regarded as a control.

The differences in PLFA composition between soil and manure were most pronounced during the first week of the experiment. Between days 1 and 3, when there was a substantial increase in total PLFA, the differences became even larger,

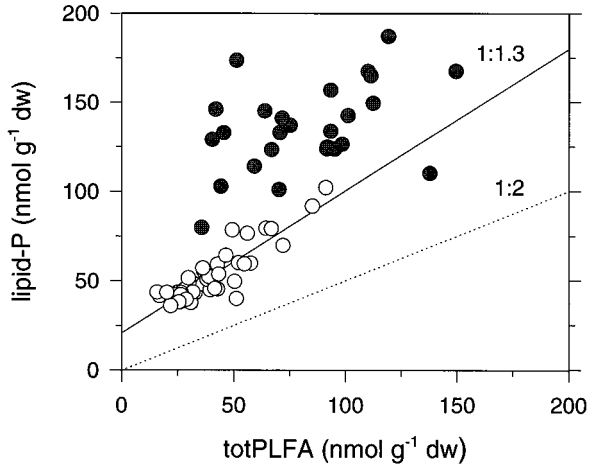

FIG. 3. Relationship between the amount of lipid-P and the total amount of PLFA (totPLFA). All sampling occasions were included. Filled symbols indicate the results for layers 1 and 2; open symbols indicate the results for layers 3 through 6. Only the results for layers 3 through 6 were included in the correlation ($n = 43$; $r = 0.89$; $P < 0.001$). The dotted line indicates the theoretical molar ratio.

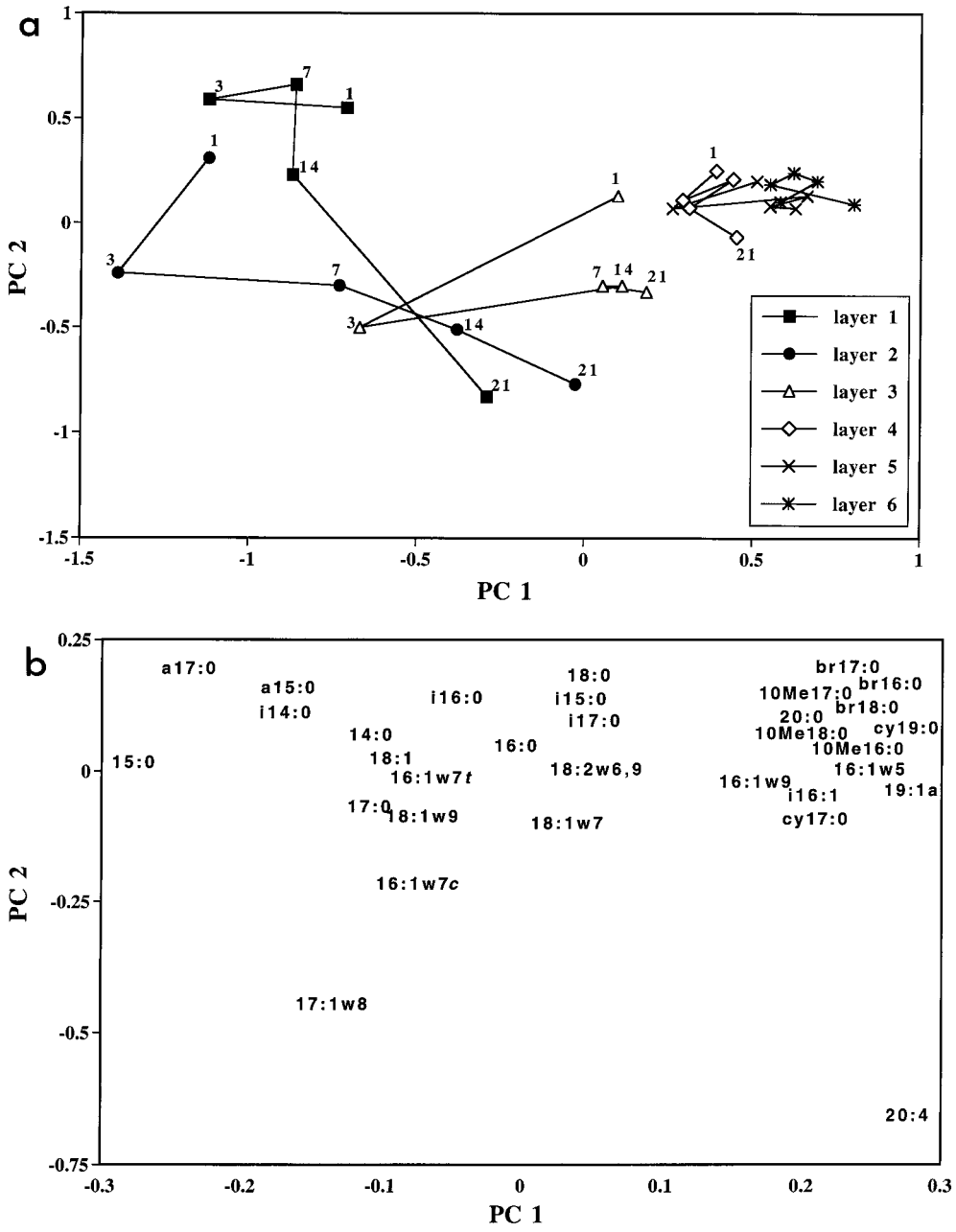

FIG. 4. (a) PC score plot showing variations in PLFA patterns in different layers in the manure hot spot after 1, 3, 7, 14, and 21 days of incubation; (b) PC loading plot showing variations in individual PLFA.

since those PLFA which were already more abundant in the manure phase increased in layers 1 through 3. This can be seen in the plot of scores (Fig. 4a), where all the samples taken on day 3 from these layers were to the left of the corresponding ones from day 1. After day 3, the PLFA composition of these layers seemed to become gradually more similar to that of the soil. In layer 3, there were only minor changes in PLFA patterns between days 7 and 21, while the PLFA patterns in layers 1 and 2 changed substantially during this period. There were smaller changes, resembling the pattern observed in layer 3, in layer 4, while the PLFA patterns in layers 5 and 6 remained unaffected throughout the incubation period. The effects on individual PLFA can be seen in the PC loading plot (Fig. 4b). The PLFA found on the left, e.g., anteisobranched a17:0 and a15:0 and saturated 15:0, were more abundant in the manure core than in the soil (Table 1). Similarly, the PLFA found on the right, e.g., methyl-branched PLFA 10Me16:0, 10Me17:0, and 10Me18:0 and three PLFA with unknown branching positions, br16:0, br17:0, and br18:0, as well as cyclopropane PLFA cy19:0 and cy17:0, were more abundant in the soil phase. The moles percent for PLFA 17:1ω8 and 16:1ω7*c*, which are found in the lower part of the loading plot (Fig. 4b), increased in the manure phase (layer 1) during the later part of the incubation period. Similarly, the amount of 20:4 increased in layers 3 and 4 during the same period.

Dynamics of individual PLFA. Although general traits can be seen from the combined PC plots, it is not possible to distinguish changes in the abundances of individual PLFA over time in different layers. Therefore, the dynamics in different layers are shown for selected PLFA (Fig. 5). In Fig. 5, the amounts of different PLFA are expressed in picomoles gram (dw) of soil^{-1}, in contrast to the PC plots, which were based on moles percent. The main changes were found in layers 1 through 3, with the other layers showing little or no change in the amounts of individual PLFA. Two general types of dynamics can be distinguished. One group consisted of PLFA which increased more or less substantially between days 1 and 3, whereupon they decreased. This group was exemplified by 15:0, 16:1ω7*c*, a17:0, and 17:1ω8 (Fig. 5A through D). In layers 2 and 3, the increases during the first days of incubation were in some cases more than threefold. For example, in layer 3, the amount of PLFA 17:1ω8 increased from 540 to 3,400 pmol g (dw)$^{-1}$ (corresponding to an increase from 1.3 to 4.2 mol%) between days 1 and 3 and the amount of 16:1ω7*c* increased from 4,600 to 15,900 pmol g (dw)$^{-1}$ (from 10.7 to 19.7 mol%) during the same period. Similar patterns were observed for several other major PLFA, e.g., i17:0, a15:0, and 18:1ω7 (not shown). The other group of PLFA consisted of those whose concentrations remained approximately the same during the first few days of incubation and thus did not show any peak on day 3. Instead, the concentrations of these PLFA increased later during incubation, as exemplified by PLFA cy17:0 and 16:1ω5 (Fig. 5E and F). Other PLFA showing similar dynamics included 10Me18:0, cy19:0, and 20:4 (for the last, see Fig. 7).

DISCUSSION

To relate the structural changes described here to different biological and physicochemical processes, Petersen et al. (20) monitored C/N turnover and oxygen uptake in the soil-manure model system in a parallel investigation. Due to the DOC concentration gradient between the two phases, there was a flux of DOC through diffusion from the manure to the soil throughout the 3-week incubation period, although it was most evident during the first week. The assimilation of DOC occurred within 10 mm of the interface. In the present investigation, the main increase in microbial biomass was found within 2 mm of the interface, but a slight increase was also seen in layers 4 and 5 (2 to 4 and 10 to 12 mm from the interface) (Fig. 2), indicating that some DOC had reached these layers. Structural changes within the microbial community were observed within the same distance from the soil-manure interface (Fig. 4). It thus appears that the changes observed in microbial biomass and community composition were closely associated with DOC turnover. The penetration of oxygen into the hot spot increased from 1 mm at the beginning of the experiment to 2 mm after 3 weeks of incubation. A transient drop in oxygen penetration was observed between days 1 and 3; it coincided with a peak in oxygen consumption (20), indicative of high microorganismal activity. During the same period, the microbial biomass, measured as total PLFA, almost doubled in the layers closest to the interface (Fig. 2).

The input rate of readily available carbon, changes in oxygen penetration, and fluxes of NH_4^+ and NO_3^- are variables likely to have influenced the composition of the microbial community associated with the manure hot spot. The accumulated oxygen respiration during the incubation period was 6.7 times higher than the NO_3^- respiration in terms of oxidizing equivalents (20); thus, aerobic respiration must have accounted for the main part of the carbon turnover in layer 2 (the outer part of the manure phase). Furthermore, nitrification was negligible during the first 3 days of incubation (20). This implies that the growth observed in layer 2 during those first days was mainly accounted for by heterotrophic, aerobic bacteria, a hypothesis supported by the similarity between layers 2 and 3 in terms of the changes that occurred in their PLFA patterns (Fig. 4 and 5); layer 3 was probably completely aerated throughout the experiment (20). As the incubation progressed, the PLFA pattern of layer 1 (the center of the manure phase) approached the PLFA pattern of layers 2 and 3 (Fig. 4), although layer 1 was anaerobic throughout the incubation period. This indicates that the decomposition of DOC was more important than O_2 availability in determining the reactions of different organisms. The shift in the PLFA composition would thus reflect the ability of different groups of microorganisms to utilize the carbon provided by the manure. However, the changes in the manure phase may also reflect the inability of certain microorganisms, many of which were probably strict anaerobes, to survive in the soil environment.

The interpretation of the changes in patterns of PLFA extracted from complex samples in terms of changes in specific taxonomic groups is complicated, since the same PLFA often exists in the membranes of organisms belonging to several different taxonomic groups. This has been discussed earlier (6). The aim of the present study was therefore to investigate the dynamics of the microbial community on a spatiotemporal level rather than to use PLFA analyses to try and pinpoint specific organisms. Some general trends, however, can be pointed out. For example, anteisobranched PLFA were more abundant in the manure phase compared with the corresponding isobranched PLFA, suggesting that the anteisobranched PLFA in this system were associated with anaerobic microorganisms. The methyl-branched PLFA found on the right in the PC plot (Fig. 4b), of which 10Me17:0 and 10Me18:0 are specific to actinomycetes (12), were not found at all in the manure (i.e., before it was mixed with the soil to be used in the experimental setup). In addition, the proportion of 16:1ω5, which has previously been reported for arbuscular mycorrhizal fungi (14) as well as for bacteria belonging to the *Cythophaga-Flexibacter* complex (13), was significantly higher in the soil than in the manure, suggesting that these PLFA were associated with aerobic organisms.

A large number of pure-culture studies have shown that factors, such as the metabolic state of the organisms, environmental changes, and toxic substances, influence the PLFA composition of cell membranes (3, 9, 22). Such effects are more difficult to show in complex environmental samples since changes in PLFA patterns have a confounding effect because of species shifts. In the present study, there must have been changes in the composition of the microbial community, at least during the early period of strong growth, since it is unlikely that all organisms competed equally well for the carbon provided by the manure and thus grew at about equal rates. However, some of the alterations observed suggest that changes occurred in membrane composition. For example, changes in the ratios of *trans* to *cis* isomers of monounsaturated PLFA, as well as changes in the ratios of cyclopropane fatty acids to their monounsaturated precursors, might have been related to the availability of DOC. These ratios were observed to increase when certain gram-negative bacteria were starved and entered a stationary growth phase (8, 10). In our model system, these ratios would thus be expected to decrease during periods of growth in layers affected by the diffusing DOC. Transient drops in these ratios were found during the period of most intense growth in layers 2 and 3 (Fig. 6) (changes in the cy17:0/16:1ω7*c* ratio showed a pattern similar to that of the cy19:0/18:1ω7 ratio [not shown]), thus suggesting that

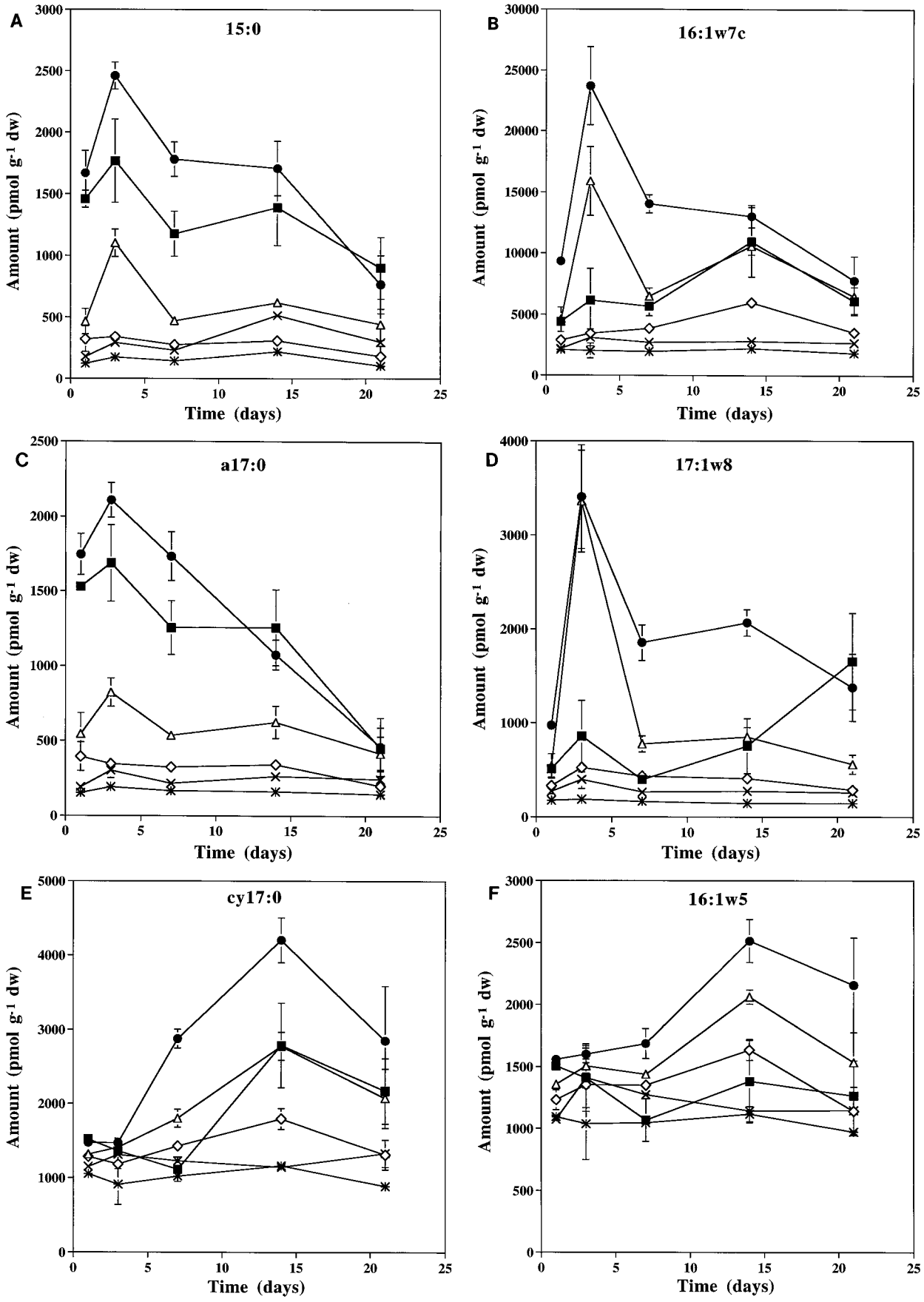

FIG. 5. Dynamics of the indicated PLFA in different layers in the manure hot spot during the 21-day incubation period. The symbols for different layers are the same as those in Fig. 4a. Bars indicate standard errors; $n = 3$ (layers 1 through 4) or 2 (layers 5 and 6).

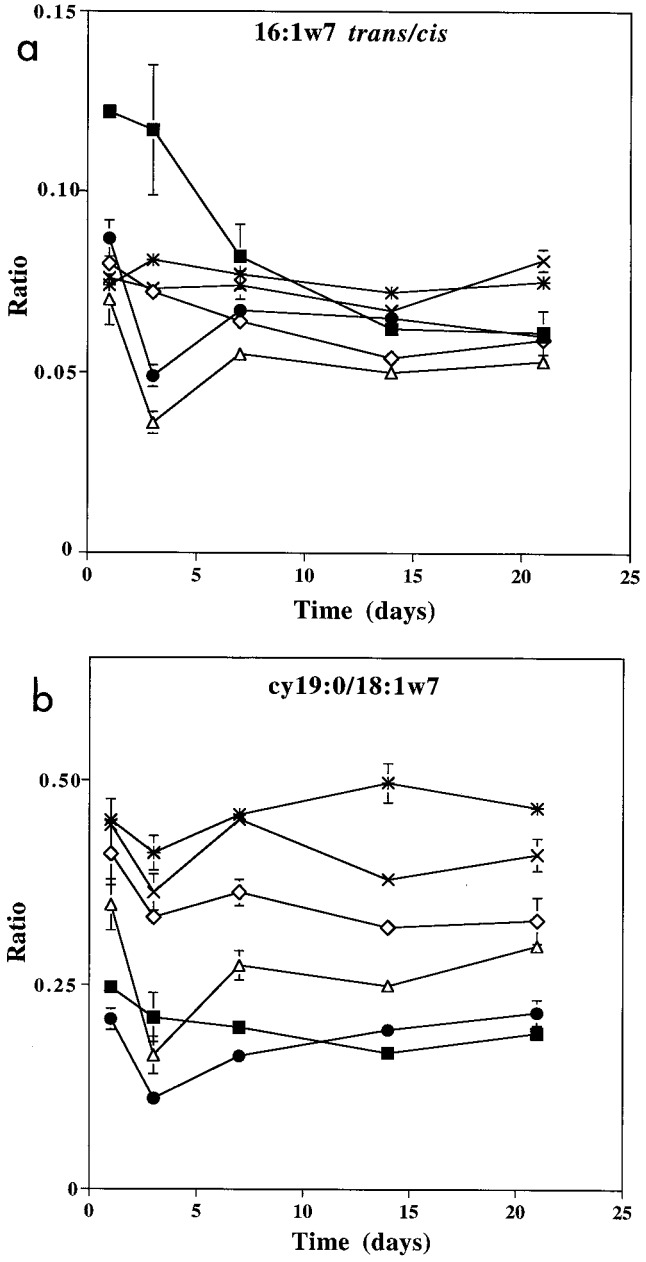

FIG. 6. Ratios of the *trans* and *cis* isomers of 16:1ω7 (a) and cy19:0/18:1ω7 (b) in different layers during the 21-day incubation period. In panel b, the sum of the *cis* and *trans* isomers of 18:1ω7 was used to calculate the ratios since they could not be separated under the GC conditions employed. The symbols for different layers are the same as those in Fig. 4a. Bars indicate standard errors; $n = 3$ (layers 1 through 4) or 2 (layers 5 and 6).

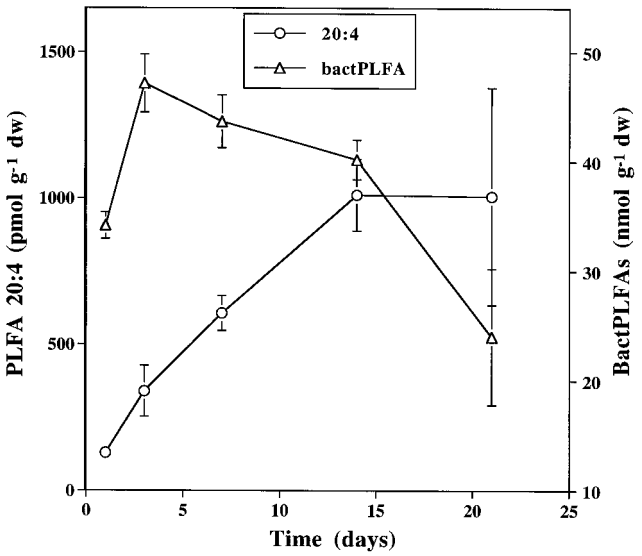

FIG. 7. Dynamics of the summed bacterial PLFA (bactPLFA) and of PLFA 20:4 in layer 2 during the 21-day incubation period.

there could have been a lower degree of isomerization during this period. In addition, the ratios of cy17:0 and cy19:0 to their precursors were highest in layers 5 and 6, which were not affected by the diffusing DOC. In contrast, the *trans*/*cis* ratio in layer 1 was higher than that in any other layer during the first week of incubation, despite the high levels of DOC in this layer. Furthermore, the sum of 16:1ω7*c* and 16:1ω7*t*, as well as the sum of cy17:0 and 16:1ω7*c* and the sum of cy19:0 and 18:1ω7, increased strongly between days 1 and 3. Thus, the changes in ratios could not be explained exclusively by alterations in membrane composition in individual organisms, since such changes would have resulted in constant values for the respective sums.

The comparison of biomass, measured as total PLFA and lipid-P, indicated the presence of a nonbiomass background of lipid-P in both the manure phase and the soil (Fig. 3). This was most pronounced for the manure phase, in which the molar ratio between lipid-P and PLFA deviated most from the expected, theoretical ratio of 1:2. There also appeared to be a background of lipid-bound phosphate in the soil phase, as indicated by the low (below the theoretical value) ratio. However, the ratio, 1:1.3, found in the soil layers (Fig. 3) is in agreement with those from other investigations (4). Elevated levels of lipid-P have also been reported in relation to biomass C, as determined by chloroform fumigation-direct extraction (19), and in relation to ATP content and substrate-induced respiration (4). It is possible that nonbiomass, lipid-bound phosphate associated with organic matter in the soil is extracted and digested along with phospholipids. This would also explain why the lipid-P values for the manure phase were comparatively higher than those for the soil phase, since the manure phase had a higher organic-matter content. It thus appears that total PLFA gives a better indication of living microbial biomass than does lipid-P. However, the rapid decreases in lipid-P and total PLFA after the first days of growth indicate that phospholipids are quickly turned over in this newly formed biomass.

Soil protozoa usually increase in number in response to an increase in soil bacteria (2). Opperman et al. (15) reported a rapid increase in the number of protozoa for the first 7 days after the addition of slurry to soil. In that study, the peak values of protozoa were reached a few days after the peak in bacteria, indicating a predator-prey relationship. Arachidonic acid (20:4) has been found in several groups of protozoa; for example, in *Paramecium* strains, this fatty acid is predominating (11). In Fig. 7, the temporal dynamics of 20:4 and the summed amount of bacterial PLFA in layer 2 are shown. The pattern obtained corresponded to the fluctuations of protozoa and bacteria described by Opperman et al. (15). The amount of bacterial PLFA increased from 34 to 47 nmol of PLFA g $(dw)^{-1}$ between days 1 and 3, after which it gradually declined

during the rest of the incubation period. In contrast, PLFA 20:4 showed a steady increase during the first 2 weeks of the experiment, reaching a maximum level 10 times higher than the initial value (Fig. 7). The amounts of bacterial PLFA and 20:4 showed a similar temporal pattern in layer 3 (not shown). Hence, the rapid turnover of microbial biomass at the soil-manure interface suggested by our results could be explained by predation.

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