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Fatty acid composition and dynamics of selected fungal-feeding nematodes and fungi

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Abstract

Fatty acid profiles of fungal-feeding nematodes, *Aphelenchus avenae* and *Aphelenchoides composticola*, and selected fungi were determined in microcosm cultures of agar, broth, or sand amended with organic matter. Fatty acids of *A. avenae* and *A. composticola* included 16:0 18:0, 18:1ω7, 18:1ω9, 18:2, 20:0, 20:1, 20:2, 20:3 and 20:4 phospholipid fatty acids (PLFAs) and neutral lipid fatty acids (NLFAs). The nematodes differed in relative amounts of saturated and C_{18} fatty acids. Similar C_{16} and C_{18} PLFAs and whole-cell fatty acids were found in *Rhizoctonia solani*, *Fusarium oxysporum* and *Trichoderma* sp. with 18:2ω6 as the major component. The C_{20} fatty acids were not found in these fungi. Although only present in the nematodes, C_{20} PLFAs were only detected when nematode population levels were ≥ 22 per gram of sand, suggesting that there is a detection threshold that might limit their use as biomarkers in the soil community. After removal of nematodes from a food source, the relative amount of C_{20} PLFAs (structural components of nematode cell membranes) decreased more slowly than the C_{16} and C_{18} PLFAs, which may have reflected ingested fungal cytoplasm in the nematode intestine. In the early stage of organic matter decomposition, total and fungal PLFAs were lower in the presence of *A. composticola* then in its absence at C:N ratios $\geq 30:1$.

Keywords: *Aphelenchus avenae*; *Aphelenchoides composticola*; Fatty acid; Fungal-feeding nematode; Fusarium oxysporum; Phospholipid fatty acids; *Rhizoctonia solani*; *Trichoderma* sp.

1. Introduction

One approach to analysis of the diversity and size of the soil community is to measure biochemical components specific to living organisms. Phospholipid fatty acids (PLFAs) are components of cell membranes and have been used to identify and quantify specific groups of soil microorganisms (Vestal and White, 1989; Frostegård and Bååth, 1996; Bossio and Scow, 1998). Neutral lipid fatty acids (NLFAs) are predominantly storage lipids and often constitute $> 70\%$ of the total lipid content (Krusberg et al., 1973; Chitwood, 1998; Fitters et al., 1999). The fatty acid data for nematodes are mainly for bacterial-feeders or parasites of vertebrates, invertebrates and plants (Krusberg, 1971; Hutzell and Krusberg, 1982; Kapur and Sood, 1995; Holz et al.,

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1997; Patel and Wright, 1997; Chitwood, 1998). There are few data available on the fatty acid composition of fungal-feeding nematodes.

Aphelenchus avenae and *Aphelenchoides composticola* are widely-distributed fungal-feeding nematodes that may have biological control potential for certain plant-parasitic soil fungi (Gupta, 1986; Ishibashi and Choi, 1991). These species also mineralize nitrogen and therefore may enhance soil fertility (Trofymow and Coleman, 1982; Ingham et al., 1985; Chen and Ferris, 1999, 2000). If the fatty acid composition of these nematodes is unique, it might provide a bioindicator of their presence and abundance. In these studies, we used microcosm systems in which the nematodes fed on *Rhizoctonia solani* or *Fusarium oxysporum*, good hosts, and *Trichoderma* sp., a poor host (Chen and Ferris, 2000) in broth, agar, or sand amended with organic mater. Our objectives were to determine: (1) major PLFAs and/or whole-cell fatty acids of *R. solani*, *F. oxysporum*, and *Trichoderma* sp.; (2) the PLFA and NLFA composition and dynamics of *A. avenae* and *A. composticola* feeding on *R. solani* and after removal from the fungus; and (3) profiles and dynamics of PLFAs in fungi and nematodes during decomposition of organic matter.

2. Methods and materials

2.1. Broth or agar cultures of nematodes and fungi

Rhizoctonia solani was cultured on Czapex Dox broth (CDB), potato dextrose broth (PDB), Sabouraud dextrose broth (SAB), tryptic soy broth (TSB), and potato dextrose agar (PDA). *Fusarium oxysporum* was cultured on PDA. The fungi and the fungal-feeding nematodes, *Aphelenchus avenae* and *Aphelenchoides composticola*, were isolated from field soils of the Sustainable Agriculture Farming System (SAFS) project at the University of California, Davis. Fungi were provided by Dr Ariena Van Bruggen, Department of Plant Pathology, University of California, Davis. Voucher specimens of the nematodes are deposited in the University of California Davis Nematode Collection with the following accession numbers: *A. composticola*-UCDNC 3640-3642, and *A. avenae*-UCDNC 3643-3644.

A 1-cm fungal plug was placed in a 250-ml flask with 100 ml broth or on the surface of a 10-cm dish with PDA. Fungi in liquid culture were grown for 4 days at 24°C on a shaker rotating at 125 rev./min. Fungal hyphae were harvested by pouring the broth through a vacuum-filter funnel with distilled deionized water (ddH_2O) (Chen and Ferris, 1999). Fungi on solid media were grown for seven days at room temperature in the dark. The agar was melted in a water bath at 60°C, and fungal hyphae were collected by the vacuum-filter funnel method used for broth.

Nematodes were cultured on *R. solani* on PDA in 10-cm Petri dishes at room temperature in the dark for 14–21 days before extraction of fatty acids. The experiment was repeated and data were pooled. Each nematode sample was collected from three Petri dish cultures by Baermann funnel extraction at room temperature for 8 h (Chen and Ferris, 1999). A nematode pellet was obtained by centrifugation at $750 \times g$ for 30 s, 30 s and 2 min; where ddH_2O was added and supernatant was discarded each time. Pelletized nematodes were stored in a freezer (−20°C). The same method was applied in gut-elimination experiments which were conducted to distinguish between PLFAs that were components of the nematode body and those that were contained in intestinal contents. Thus, one-third of the nematode samples obtained by Baermann funnel extraction was processed immediately by centrifugation and freezing. The remainder was placed in beakers with ddH_2O and aerated in an incubator for food-deprivation periods of 48 or 96 h at 16°C to allow the nematodes to utilize or eliminate gut contents. There were four replications of each treatment.

2.2. Nematode and fungal cultures on sand amended with organic matter

Sand (particle size distribution: 0.6% 1.0 mm; 8.5% 0.5 mm; 54.2% 0.25 mm; 32.4% 0.10 mm; 1.1% 0.05mm; 1.8% silt; 1.3% clay) was water-rinsed, acid-washed, and water-rinsed to remove organic materials (Chen and Ferris, 1999). The sand was autoclaved twice for 4–6 h at 24-h intervals before addition of organic matter. The nitrogen-free sand (450 g) was amended with 1 g of alfalfa hay (C/N = 11:1), combined with fibrous cellulose powder (W. and R. Balston, Ltd., England) to create four C/N ratios, 11:1, 20:1, 30:1 and 40:1, and inoculated with fungi alone or

with fungi and nematodes (Chen and Ferris, 1999). Polyvinyl chloride tubes (32 cm long) were closed at one end with caps in which a 1-cm diameter hole had been drilled. Under sterile conditions the tubes were packed with the amended sand and placed in boxes to minimize contamination. The microcosm system was fungal-dominated, suggested by the fact that only approximately 18% of total PLFAs on day 21 were bacterial.

Three replications were established for each nematode-fungus-C:N ratio combination. Columns with *R. solani*, with and without *A. composticola*, were destructively sampled on day 0, 7, 14 and 21 for PLFA analysis. Approximately 100 g sand from each column was collected and stored in a freezer (−20°C). On day 21, several other fungus/nematode combinations also were sampled, including: (1) *R. solani* with and without *A. avenae*; and (2) *Trichoderma* sp. with and without *A. avenae* or *A. composticola*. Nematode population level was determined by the following procedure and adjusted for extraction efficiencies of 79.3% for *A. avenae* and 83.5% for *A. composticola* (Chen and Ferris, 1999). An approximately 0.5-cm layer of sand (50 g) was placed on a Whatman No. 4 filter paper supported by a concave screen (8 cm diameter) and partially submerged in water in a Baermann funnel. A 50-ml suspension was collected from the bottom of the funnel system 24 h later. Nematode numbers in three replicates of 1 ml of the suspension were counted.

2.3. Extraction of fatty acids

Sand moisture level was determined so that a standardized weight of sand could be used in each extraction. Samples of 8 g dry weight equivalent of sand, or nematode biomass harvested from PDA agar culture, were extracted by shaking a 23-ml mixture of chloroform/methanol/phosphate buffer (1:2:0.8) for 2 h. Samples were then centrifuged at $904 \times g$ for 10 min. The supernatant was transferred to a separatory funnel and the sample was re-extracted for 30 min using an additional 23 ml of the buffer mixture. After centrifugation, the supernatant from the second extraction was added to the first along with 12 ml of buffer and 12 ml of chloroform. The samples were shaken and allowed to stand overnight. The lower layer in the separatory funnel was drained, then dried at 32°C with nitrogen. Solid phase extraction columns, 0.50 g Si (Supelco, Inc., Bellefonte, PA) were used to separate the samples into three major lipid classes, neutral lipids, glycolipids and phospholipids using chloroform, acetone and methanol, respectively. The collected fractions were then dried at 32°C with nitrogen. Fatty acid methyl esters (FAME) were formed at 37°C using 1 ml 0.2 M KOH and 1 ml of a mixture of methanol/toluene (1:1). After 15 min the FAMEs were extracted twice with 2 ml hexane and 2 ml water. Samples were analyzed using a Hewlett Packard 6890 gas chromatograph with a 25 m × 0.20 mm Ultra2 column. FAMEs were identified using standards and MIDI peak identification software (MIDI, Inc., Newark, DE). FAME identities were confirmed using a Finnigan GCQ (ThermoQuest Co., San Jose, CA) in both EI mode and CI mode with acetonitrile as the reagent gas.

Fungal biomass was also harvested from broth or agar cultures for whole-cell fatty acid composition analysis. Samples were saponified using 3.75 M NaOH in methanol at 100°C for 1 h. Then 3.25 M HCl in methanol was added to methylate the samples at 80°C. The resulting FAMEs were extracted using hexane/methyl-*tert*-butyl ether (1:1). Samples were analyzed as above.

2.4. Data interpretation

Fatty acids were identified according to the number of carbon atoms, amount and position of unsaturation, and functional group substitution. The percentage distributions of major PLFAs, NLFAs and whole-cell fatty acids recovered from nematodes and fungi were calculated. Individual fatty acids were measured as pmol/g dry sand. The size of the fungal biomass was indicated by weight of fatty acids attributed to fungi (Bossio and Scow, 1998; Mikola and Setala, 1998). Correspondence analysis (CA), a multivariate ordination technique, was applied to graphically group and summarize PLFA and NLFA data for two nematode species (Jongman et al., 1995).

3. Results

3.1. Fungal fatty acid composition

Four major whole-cell fatty acids were recovered from *R. solani* regardless of the growth

Table 1
Percentage distribution (mean ± standard error, $n = 3$, based on molarity) of major whole-cell fatty acids recovered from *Rhizoctonia solani* and *Fusarium oxysporum* cultured on broth or agar media

Fatty acid	*Rhizoctonia solani*				*Fusarium oxysporum*
	PDB	SAB	CDB	TSB	PDA
16:0	16.3 ± 4.0	11.7 ± 2.1	13.7 ± 5.2	6.1 ± 1.8	24.7 ± 0.1
18:0	7.2 ± 1.3	5.1 ± 0.7	9.5 ± 1.0	0.6 ± 0.6	8.7 ± 0.7
18:1ω9	10.3 ± 1.9	5.8 ± 1.0	16.2 ± 1.2	5.5 ± 1.4	30.6 ± 0.7
18:2ω6	46.9 ± 4.8	60.5 ± 9.3	45.4 ± 0.6	56.0 ± 11.5	32.6 ± 0.8

Abbreviations: PDB, potato dextrose broth; SAB, Sabouraud dextrose broth; CDB, Czapex Dox broth; TSB, tryptic soy broth; PDA, potato dextrose agar.

medium on which the fungus was cultured: 16:0, 18:0, 18:1ω9 and 18:2ω6 (Table 1). These four fatty acids accounted for 81%, 80%, 85% and 68% of total fatty acids recovered from PDB, SAB, CDB and TSB cultures, respectively. On the average across the four broth media, fatty acid 18:2ω6 accounted for 52.2% ± 3.8 of total fatty acids. The second most common fatty acid was 16:0 which comprised 12.0% ± 2.2 of total fatty acids. In *F. oxysporum* culture, the four major fatty acids accounted for 72% of total whole-cell fatty acids recovered from PDA. Fatty acids 18:2ω6 and 18:1ω9 accounted for 33% and 31% of total fatty acids, in comparison with fatty acid 16:0 which comprised approximately 25% of the total (Table 1). On a weight basis, extracts of both *F. oxysporum* and *R. solani* contained more unsaturated than saturated fatty acids.

3.2. Fatty acid composition and their dynamics in nematodes

Fatty acids recovered from *A. avenae* and *A. composticola*, extracted from *R. solani* cultures on PDA, included 16:0 18:0, 18:1ω7, 18:1ω9, 18:2, 20:0, 20:1, 20:2, 20:3 and 20:4 PLFAs and NLFAs. (Table 2). Percentage distribution of unsaturated C_{20} fatty acids generally did not differ between

Table 2
Fatty acid composition (mean percent ± standard error, $n = 6$, based on molarity) of fungal-feeding nematodes

Fatty acid	*Aphelenchus avenae*		*Aphelenchoides composticola*	
	PLFA[a]	NLFA[b]	PLFA	NLFA
14:0	ND	3.1 ± 0.6	ND	2.8 ± 0.5
15:0 *iso*	ND	1.9 ± 0.3	ND	2.6 ± 0.7
16:0	4.8 ± 0.5	12.0 ± 0.2	7.0 ± 2	9.9 ± 0.3
16:1ω7	ND	1.9 ± 0.3	ND	0.3 ± 0.1
17:0 *iso*	ND	1.1 ± 0.3	ND	2.3 ± 0.4
17:0	ND	0.2 ± 0.6	ND	0.9 ± 0.2
18:0	18.0 ± 0.6	3.8 ± 0.3	24.9 ± 0.7	6.1 ± 0.4
18:1ω7	7.2 ± 0.6	15.4 ± 1	2.7 ± 1	1.0 ± 0.4
18:1ω9	11.5 ± 0.8	25.2 ± 0.7	14.8 ± 0.5	25.5 ± 3
18:2	27.2 ± 2	15.3 ± 0.3	15.5 ± 1	11.4 ± 1
20:0	5.7 ± 0.2	0.5 ± 0.02	2.8 ± 0.2	0.5 ± 0.03
20:1	3.2 ± 0.3	2.7 ± 0.2	5.7 ± 0.4	2.5 ± 0.3
20:2	0.9 ± 0.2	0.5 ± 0.04	1.4 ± 0.7	0.4 ± 0.03
20:3	7.7 ± 1	1.2 ± 0.1	8.5 ± 2	1.5 ± 0.1
20:4	11.7 ± 2	3.0 ± 0.2	10.8 ± 2	2.3 ± 0.2
Sum	**97.9**	**87.8**	**94.1**	**70.0**
Monounsaturated	21.9	45.2	23.2	29.3
Polyunsaturated	47.5	20.0	36.2	15.6
Saturated	28.5	22.6	34.7	25.1

[a] *Abbreviations:* PLFA, phospholipid fatty acid; ND, no detection or not detected from all samples.
[b] NLFA, neutral lipid fatty acid. The list does not contain NLFAs that are not detected from all samples.

Fig. 1. Correspondence analysis for fatty acid profiles of *Aphelenchus avenae* (AA) and *Aphelenchoides composticola* (AC). Profiles are generally grouped by the species and fractions of phospholipid (PL) and neutral lipid (NL), with the largest variance in PLFAs of *A. composticola*.

the two species. However, percentage distributions of C_{16} and C_{18} PLFAs were different for the two fungal-feeding nematodes. The two species also differed in polyunsaturated and saturated C_{18} PLFAs, and in position of the double bond of the PLFA 18:1.

The fatty acid data were analyzed by CA and were found to group by species and fractions of PLFA and NLFA (Fig. 1). Fatty acid profiles of the two nematode species differed, but less than the differences between the two lipid fractions. All samples of *A. avenae* are above the horizontal axis, while all of the *A. composticola* are below the axis. Of all PLFAs, 32% were saturated, compared with 16% of the NLFAs (Table 2). The percent of saturated C_{20} fatty acids was greater for PLFAs than for NLFAs. On a weight basis, monounsaturated fatty acids were lower and polyunsaturated fatty acids were higher among PLFAs than NLFAs.

During the first 48 h of food deprivation, the relative amount of PLFAs in nematodes with 16 and 18 carbons decreased (Fig. 2). During the next 48 h, the relative amount of PLFAs with 20 carbons decreased. The PLFA 16:0, 18:1ω7, 18:2, and unsaturated C_{20} were those most affected, while others were relatively unchanged as food-deprivation time increased.

3.3. PLFA profiles during organic matter decomposition by R. solani with and without nematodes

Sand cultures with organic matter, inoculated with fungi and with or without nematodes, mainly contained PLFAs 16:0, 18:0, 18:1ω9 and 18:2ω6 (Fig. 3b, Table 3). As in broth or agar cultures, there were greater amounts of unsaturated than saturated fatty acids. In sand amended with *R. solani* in the absence of nematodes, 18:2ω6 accounted for 12.2% and 18:1ω9 accounted for

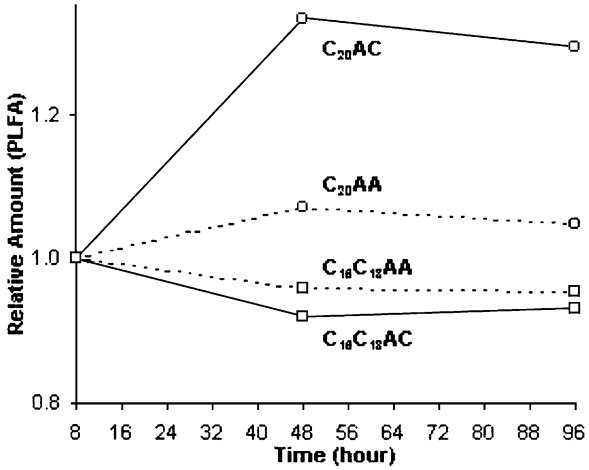

Fig. 2. Dynamics of PLFAs in fungal-feeding nematodes during food deprivation. The *Y*-axis indicates relative amounts of PLFAs. C_{20}, PLFAs with 20 carbons; $C_{16}C_{18}$, PLFAs with 16 or 18 carbons; AA, *Aphelenchus avenae*; AC, *Aphelenchoides composticola*.

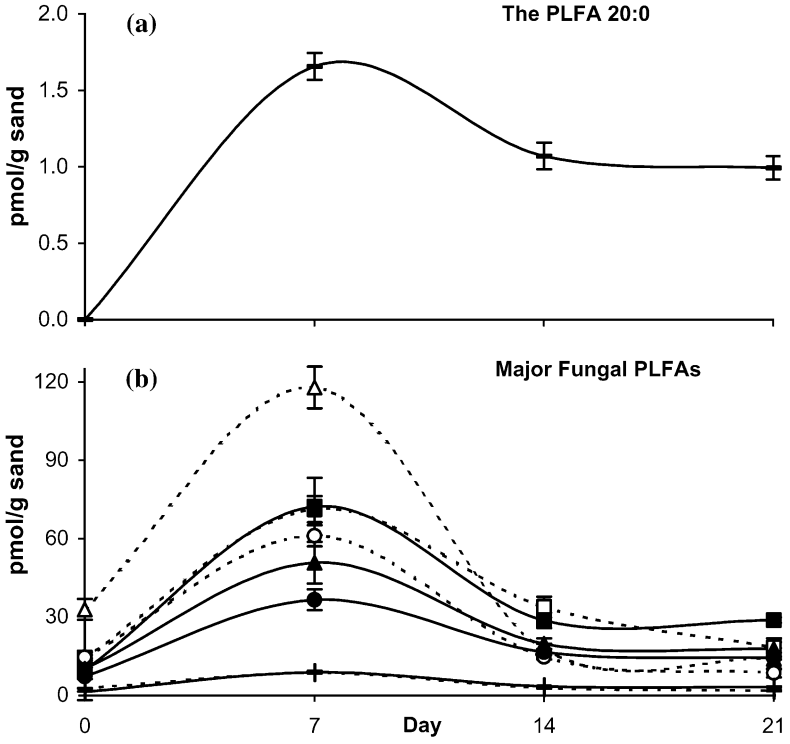

Fig. 3. Effect of fungal-feeding nematodes on fungal PLFAs during organic matter decomposition in columns colonized by *Rhizoctonia solani* and *Aphelenchoides composticola.* Bars indicate standard errors. Some bars are obscured by symbols. (a) Dynamics of the PLFA 20:0, (b) dynamics of the PLFA 16:0 (square), 18:0 (diamond), 18:1ω9 (triangle), and 18:2ω6 (circle). Dotted lines and empty symbols indicate the absence of nematodes. Solid lines and solid symbols indicate the presence of nematodes.

23.5% of total PLFAs on day 7. On day 21, 18:2ω6 accounted for 10.2% and 16:0 accounted for 14.8% of total PLFAs.

The fatty acid composition of *R. solani*-inoculated microcosms differed depending on whether nematodes were present or not. C_{20} PLFAs were not detected from samples with organic matter and fungus only. The C_{20} PLFAs were also not detectable in microcosms with *Trichoderma* sp. regardless of whether nematodes were present

Table 3
PLFA profiles (mean pmol/g sand or percentage of total PLFA) after 21 days of organic matter decomposition

PLFA	RS[a]	RS + AA	TS	TS + AA	RS	RS + AC	TS	TS + AC
Total	149	196	112	119	142	178	136	133
16:0	24 (16.4)	34 (17.4)	21 (19.2)	26 (21.4)	18 (13.1)	29 (16.2)	27 (20.1)	24 (17.8)
18:0	3 (2.1)	4 (2.1)	2 (1.4)	2 (1.8)	2 (1.3)	3 (1.8)	2 (1.7)	2 (1.5)
18:1ω9	12 (7.7)	14 (6.9)	16 (14.1)	15 (12.4)	14 (9.9)	18 (10.0)	20 (14.3)	17 (12.4)
18:2ω6	14 (9.2)	14 (7.3)	14 (12.9)	11 (9.4)	9 (6.0)	14 (8.1)	22 (15.9)	17 (12.5)
20:0[b]	ND	ND	ND	ND	ND	0.99	ND	ND
20:4[c]	ND	0.63	ND	ND	ND	ND	ND	ND
Unsaturated	51 (34.1)	75 (38.3)	45 (40.1)	44 (36.8)	67 (47.4)	82 (46.4)	68 (49.9)	55 (41.2)
Saturated	30 (20.3)	42 (21.3)	25 (22.3)	30 (24.7)	23 (15.9)	35 (19.9)	30 (22.0)	26 (19.5)

[a] *Abbreviations:* RS, *Rhizoctonia solani*; AA, *Aphelenchus avenae*; TS, *Trichoderma* sp.; AC, *Aphelenchoides composticola*. Percentages of total PLFA in brackets. No nematodes were recovered from samples with fungus only. The mean nematode population densities per gram of sand were 22 for RS + AA, 10 for TS + AA, 48 for RS + AC and 12 for TS + AC, respectively. The PLFAs 20:1 and 20:2 were detected (one of eight samples) on day 21 (0.73 pmol/g sand, RS + AA) and day 7 (0.12 pmol/g sand, RS + AC), respectively.

[b] Standard error = 0.08 pmol/g sand, $n = 8$, RS + AC. ND, no detection.

[c] Standard error = 0.04 pmol/g sand, $n = 8$, RS + AA.

(Table 3). The mean nematode population densities were 10–12 individuals per gram of sand in the presence of *Trichoderma* sp. (a poor host), and 22–48 in the presence of *R. solani* (a good host). In the presence of *A. composticola* and *R. solani*, there were 0, 1.66, 1.07, 0.99 pmol/g sand of the PLFA 20:0 on day 0, 7, 14, and 21, respectively (Fig. 3a). The PLFA 20:2 was detected (0.12 pmol/g sand) on day 7. Samples taken on day 21 were analyzed for other nematode-fungal combinations and there was 0.63 pmol/g sand of the PLFA 20:4 in the presence of *A. avenae* and *R. solani*. There also was 0.73 pmol/g sand of the PLFA 20:1 (Table 3).

The presence of *A. composticola* resulted in lower amounts of the PLFA 18:1ω9 and 18:2ω6 in the early stage of organic matter decomposition than with fungus only. By day 14, there was no longer an effect of the nematode (Fig. 3b). When the C/N ratio of the organic matter was high ($\geq$ 30:1), the nematode was associated with significant reductions in major and total PLFAs extracted from columns on day 7 (Table 4). By day 14, both major and total PLFAs in the columns had declined in all treatments and nematode effects were no longer evident.

4. Discussion

4.1. Nematode and fungal fatty acids

Aphelenchus avenae and *A. composticola* are representative species of fungal-feeding nematodes and are widely distributed in soils. The major PLFAs and NLFAs of the two species contain 18 or 20 C atoms, with 18:2 and 18:1 the most abundant. The major fatty acids recovered from the fungi, *R. solani*, *F. oxysporum*, and *Trichoderma* sp., are marker PLFAs for fungi (Vestal and White, 1989), and 18:2ω6 and 18:1ω9 have been used as reliable estimators for fungal biomass (Mikola and Setala, 1998). These fungal PLFAs and whole-cell fatty acids, regardless of culture media, do not contain C_{20}. Similar observations have been reported elsewhere for fungi (Weete, 1980; Johnk and Jones, 1992, 1993, 1994; Hering et al., 1999).

Our results are generally in agreement with previous studies for nematodes. Womersley et al. (1982) reported that 18:2 and 18:1 accounted for 41% and 31%, respectively, of total fatty acids in anhydrobiotic larvae of *A. avenae*. *Panagrellus redivivus*, feeding predominantly on yeast, contains 18:2 as its major fatty acid component (Silvapalan and Jenkins, 1966). Krusberg (1967) reported that the major fatty acid component is 18:1 for several plant-feeding nematodes, including *A. ritzemabosi*, *Ditylenchus dispsaci* and *D. triformis*, the last of which was cultured on the fungus *Pyrenochaeta terrestris*. A wide range of nematodes, bacterial and fungal feeders, and parasites of plants or animals, contain C_{20} fatty acids (Krusberg, 1967; Kapur and Sood, 1995; Chitwood, 1998).

The fatty acid composition of fungal-feeding nematodes in this study indicated interesting differences from those reported for some plant-parasitic nematodes. Krusberg et al. (1973) found that neutral lipids (storage lipids) constituted 90% of the total lipids of *Meloidogyne* females while phospholipids constituted 9%. Only trace amounts of the NLFAs of those nematodes were polyunsaturated fatty acids while 24–30% of the PLFAs

Table 4
Effect of C/N ratio of microcosm substrate on amounts of major and total phospholipid fatty acids (PLFAs) in the presence of *Rhizoctonia solani* (RS) during organic matter decomposition with and without *Aphelenchoides composticola* (AC)

	Day 7		Day 14		Day 21	
C/N	$\leq$ 20:1	$\geq$ 30:1	$\leq$ 20:1	$\geq$ 30:1	$\leq$ 20:1	$\geq$ 30:1
Major[a]						
RS	270 ± 26	240 ± 22	80 ± 9	60 ± 1	50 ± 13	40 ± 3
RS + AC	210 ± 29	130 ± 2	60 ± 4	70 ± 14	60 ± 1	70 ± 1
Total						
RS	530 ± 52	480 ± 38	250 ± 49	200 ± 15	160 ± 28	120 ± 17
RS + AC	450 ± 6	200 ± 9	170 ± 19	190 ± 44	170 ± 8	190 ± 1

[a] Major PLFAs (mean + standard error, pmol/g sand) consist of 16:0, 18:0, 18:1ω9 and 18:2ω6.

were polyunsaturated. However, Holz et al. (1998) reported that potato cyst nematode NLFA consisted of 42% of polyunsaturated fatty acids. Although we are unable to calculate the percentage contribution of NLFAs and PLFAs to total lipids, having discarded the glycolipid fraction during processing, the polyunsaturated fatty acids were in the range of 16–20% of the NLFAs and 36–48% of the PLFAs. High levels of polyunsaturated fatty acids were also found in the neutral lipid fraction of *P. redivivus* feeding on yeast (Silvapalan and Jenkins, 1966). It seems that polyunsaturated NLFAs represent a substantial component of total lipids in fungal-feeding nematodes and differ in amounts between cyst and root-knot nematodes, two important groups of plant-feeders.

Krusberg et al. (1973) indicated, that in *Meloidogyne*, the 18:1 content of the NLFAs was approximately 25% higher than that of PLFAs. Our results indicate differences in the same order of magnitude. A striking difference between our data for the fungal-feeding nematode and those reported elsewhere for other nematodes is in the ratio of vaccenic acid (18:1ω7) to oleic acid (18:1ω9). In other studies that we have seen, 18:1ω7 is an equal to much larger percentage of the total fatty acids than is 18:1ω9 (Krusberg, 1972; Krusberg et al., 1973; Hutzell and Krusberg, 1982; Tanaka et al., 1996). In our study, 18:1ω9 constituted a larger percentage of total PLFAs and NLFAs than 18:1ω7. In an early report on fatty acid composition of *Turbatrix aceti*, Rothstein and Götz (1968) suggested that 18:1ω9 is the most prevalent fatty acid. However, Krusberg (1972) repeated the fatty acid analyses of *T. aceti* and concluded that 18:1ω9 and 18:1ω7 were present in equal amounts. Krusberg concluded that the lower ratio of 18:1ω7 to 18:1ω9 in *T. aceti* was due to a reduction of 18:1ω7 as the amount of 18:1ω9 was consistent with that reported in other studies. In fungal-feeding nematodes, the percentage of 18:1ω9 is similar to that reported in *T. aceti* (Krusberg, 1972) while the percentage contribution of 18:1ω7 to total fatty acids is lower.

Although 20:5 fatty acids constituted 9.6–15.7% of the PLFAs of root-knot nematodes (Krusberg et al., 1973) and are the predominant C_{20} PLFAs in some bacterial-feeding nematodes (Silvapalan and Jenkins, 1966; Fodor et al., 1994; Tanaka et al., 1996), we did not detect them among either PLFA or NLFA fractions. The total percentage of C_{20} PLFAs in our study was 29.2 for both fungal-feeding nematodes. For plant-feeding nematodes it was 24.4% for *M. incognita* and 33.0% for *M. arenaria* females (Krusberg et al., 1973). In both studies, the C_{20} fatty acids represent a substantial component of total PLFAs. C_{20} fatty acids were found in *Steinernema carpocapsae* and *Caenorhabditis elegans* even when those fatty acids were not detectable in the culture media, leading to speculation of de novo biosynthesis of long-chain unsaturated fatty acids by nematodes (Rothstein and Götz, 1968; Fodor et al., 1994; Tanaka et al., 1996).

Though PLFA and NLFA compositions of *A. avenae* and *A. composticola* were generally similar, relative amounts of the components differed. These differences were also evident in the correspondence analysis in which the species were discriminated more clearly by NLFAs than by PLFAs. Whether there is potential for the differences to be used as biomarkers for the individual species warrants further investigation.

We hypothesize that when deprived of fungus as food, nematodes would deplete unassimilated fungal material so that PLFAs measured subsequently represent, predominantly, nematode structure. The relative distribution of C_{16} and C_{18} PLFAs declined first, followed by the C_{20} fatty acids (Fig. 2). It is unlikely that total nematode lipids increase during food deprivation, so the relative change should represent decreases in total amounts of the individual PLFAs. That suggests that either there are differential changes in structural components of the nematode body during the early and late stages of food deprivation or that the C_{16} and C_{18} were associated with unassimilated fungus in the nematode intestine.

4.2. PLFA profiles and dynamics during organic matter decomposition by R. solani in sand microcosms with and without nematodes

Under the conditions and methods employed in this study, C_{20} PLFAs were not detected in sand samples amended with fungus only. The PLFAs 20:0, 20:1, 20:2 and 20:4 were found only in samples containing nematodes. That suggests that C_{20} fatty acids may be useful as biomarkers for nematodes in field studies. However, there was a problem of resolution in that the marker was observable only when the number of nematodes

was relatively high. At low nematode population densities, 10–12 nematodes/g sand, C_{20} PLFAs were not detected. It may be possible to improve the sensitivity of the detection method. Nevertheless, the possibility that other organisms, such as insects (Ogg et al., 1993), may contain C_{20} fatty acids also has to be considered. Due to differences in body size, such organisms could probably be separated from samples by differential extraction techniques.

Major and total PLFAs did not differ between fungal decomposition columns with and without nematodes on day 14 and 21. However, both major and total PLFAs were lower in the presence of nematodes on day 7 in columns with high C/N ratio organic material (Table 4). The difference was much less in columns with low C/N ratios. Since nematodes suppressed fungal biomass at C/N ratio > 30:1, as indicated by PLFA amount on day 7 (Table 4), we conclude that the nematode population had declined by day 14 so that the suppressive effect was no longer evident. The PLFA data suggest that fungal growth was greater on organic matter of low C/N ratios and that there was greater nematode impact when the fungus was growing more slowly on high C/N ratio organic matter in the early stage of decomposition.

Approximately 50% of the total whole-cell fatty acid of *R. solani* was 18:2ω6 in broth cultures, and approximately 10% in sand microcosms. On day 7, the amount of 18:1ω9 was the greatest among all PLFAs. On day 21, the amount of 16:0 was the greatest. 18:2ω6, 18:1ω9 and 16:0 accounted for 48% of total PLFAs in sand amended with *Trichoderma* sp. on day 21. The observed abundance of these PLFAs confirmed their potential as biomarkers for fungi and as reliable estimators for fungal biomass (Vestal and White, 1989; Frostegård and Bååth, 1996; Mikola and Setala, 1998).

PLFAs may provide a quantitative measure of fungal biomass affected by nematode grazing. As reliable conversion factors from PLFAs to total fungal biomass are lacking (Frostegård and Bååth, 1996), differences may be indicated by comparison of fatty acid weights between fungi alone and fungus plus nematode combinations. Nematodes contain a relatively high percentage of C_{18} fatty acids that are commonly used as fungal biomarkers. If nematode population densities are low in soil, their presence will have little effect on estimates of fungal biomass. However, if there is a large number of nematodes present in soil, the contribution of nematode PLFAs should not be overlooked. Otherwise, the effect of nematode grazing on fungal biomass may be underestimated.

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