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PRIMARY RESEARCH PAPER

Changes in the nutritional quality of decaying leaf litter in a stream based on fatty acid content

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Abstract We examined the nutritional quality of decaying leaf litter in a third-order forested stream, using measurements of fatty acid (FA) composition over time. We measured changes in concentrations of total, polyunsaturated, microalgal, and microbial marker FAs in mixed-species leaf packs in spring and autumn and effects of including/excluding macroinvertebrates. Initial concentrations of total FAs in litter were significantly less in spring (5.2 mg/g) than in autumn (6.9 mg/g; $F = 6.3$; $P = 0.03$), but total FA concentrations in litter placed in the stream declined significantly over 120 days in both spring (62%; $F = 10.9$; $P < 0.001$) and autumn (56%; $F = 19.4$; $P = 0.0001$). Quantities of most FAs declined at a greater rate than that of bulk leaf matter. The presence or absence of macroinvertebrates (5 mm vs. 250 μm mesh) had no effect on FA concentration or composition of decomposing litter. Omega-3 polyunsaturated FAs were either nearly absent (20:5ω3) or depleted preferentially over other FAs (18:3ω3). During decomposition the polyunsaturated FA linoleic acid (18:2ω6, common in fungi), declined in concentration more rapidly than other FAs in the spring, but in autumn declined at slower rates, perhaps suggesting greater fungal activity in autumn. Quantities of bacterial (e.g., 16:1ω7) and fungal (e.g., 18:1ω9) FA markers increased over time in autumn (and 16:1ω7 also in spring). Our data provide no evidence for increasing nutritional FA quality of litter during decay and microbial colonization, based on total and polyunsaturated FAs, despite measured increases in bacterial and fungal FA over time. Routine measurements of FA composition of litter could provide insights into the nutrition of allochthonous matter and the importance of fungi and bacteria during decomposition.

Keywords Allochthonous matter · Leaf litter · Fatty acids · Decomposition · Stream · Macroinvertebrates · New York · Nutrition

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Introduction

Allochthonous material in the form of autumn-shed leaves is generally thought to constitute the largest carbon source for temperate, low-order stream food webs (Cummins & Klug, 1979; Vannote et al., 1980). It is well established that annual quantities (as carbon) of leaf litter falling into streams typically far exceed algal biomass produced within most forested streams (Methvin & Suberkropp, 2003). There have been many studies on the fate and

importance of leaf litter in streams (Suberkropp et al., 1976; Boulton & Boon, 1991). However, the nutritional quality of this food source may render it less important for benthic consumers. Nutrient retranslocation prior to leaf fall is thought to make autumn-shed-leaf detritus poor in nutrients, such as nitrogen (Killingbeck, 1996; Sterner & Elser, 2002). Nutrient content of leaf litter may later be increased by fungi and bacteria, which colonize leaves before and after entering the stream channel (Barlocher & Kendrick, 1974; Triska & Sedell, 1976). For this reason, the "peanut butter on crackers" analogy has often been used to characterize microbial biofilms on lower quality leaf litter (Cummins, 1974). Similarly, it has been suggested that the nutritional quality of allochthonous carbon in lake food webs may somehow be enhanced by microbial processing (Matthews & Mazumder, 2006), although the mechanisms of this reformulation are not well studied.

There is ample evidence that bacterial and fungal biomass increase on leaf litter during decomposition and conversion of CPOM to FPOM (Findlay & Arsuffi, 1989). Rates of fungal and bacterial colonization of leaf litter can be affected by several seasonally varying factors, such as temperature, as evidenced by greater biomass and production of decomposing fungi in the autumn than in the spring (Methvin & Suberkropp, 2003). The rate of leaf processing is also a function of N content, and is often predicted based on C:N ratios (e.g., Webster & Benfield, 1986; Gessner et al., 1999). Results from macroinvertebrate feeding experiments clearly indicate the importance of microbes on decomposing leaves for consumers. This process, often termed conditioning, is described as an increase in leaf litter palatability and nutritional value due to softening, fragmentation, mineralization, and microbial colonization (Gessner et al., 1999). Colonized or conditioned detritus is more readily eaten and assimilated by stream invertebrates and promotes better growth than sterile leaf material (Barlocher & Kendrick, 1975; Fuller & Fry, 1991). Microbial lipids could be responsible for this preference (Cargill et al., 1985a, b).

The change in lipid content of leaf litter with decomposition and colonization has been little studied as compared with changes in C, N, or P content. In a woodland stream Suberkropp et al. (1976) observed that total lipid decreased in leaf litter over time at rates intermediate between soluble compounds and structural components. On the other hand, Mfilinge et al. (2003) observed an increase in total lipids and fatty acids (FAs) with decomposition of mangrove leaves. An important part of the lipid content of leaves is its FA fraction. The supply of polyunsaturated fatty acids (PUFAs) to aquatic invertebrates is essential and can limit consumer secondary production because PUFAs are metabolically required and cannot be synthesized by the consumer (Muller-Navarra et al., 2000; Arts et al., 2001). A recent study by the authors has shown experimentally that a common stream invertebrate, the caddisfly *Hydropsyche* sp., must acquire PUFAs from their diet and cannot synthesize many of these essential FAs (Torres-Ruiz et al., 2010). Despite the potential importance of PUFAs for stream consumers and the often-cited significance of leaf litter in the diet of invertebrates in small streams (Vannote et al., 1980), only one study is known that addresses the subject of FA dynamics in leaves during decomposition in streams. Mills et al. (2001) determined that PUFAs (mainly 18:2ω6) present in sweetgum (*Liquidambar styraciflua*) and water oak (*Quercus nigra*) leaves were preferentially degraded relative to bulk leaf matter in a blackwater stream. In general, live terrestrial leaf matter is characterized by high levels of 18-C PUFAs (e.g., 18:2ω6, 18:3ω3), non-detectable levels of C20-PUFAs, and mid levels of long-chain saturated FAs (e.g., 24:0), which are part of cuticular waxes (Napolitano, 1999). Their fate in detrital food webs within streams is thus far unknown.

FA composition of fungi and bacteria colonizing leaf matter in fresh waters is also insufficiently known. Nevertheless, studies on fungi from terrestrial and marine environments have identified certain characteristic FAs. The most common FAs reported from more than 100 fungal species were 16:0, 18:0, 18:1ω9, and 18:2ω6, but very low (<5%) amounts of C20-PUFAs (Cooney et al., 1993; Stahl & Klug, 1996). Although 18:2ω6 can be present in green plants and green algae, it may be considered a fungal marker in studies of autumn-shed leaves and detritus, because leaves have lost most of this FA prior to leaf fall (i.e., leaves are dead). The FA 18:1ω9 is also abundant in many living organisms but is also rapidly lost after death. Bacterial lipids and FAs have been widely used to assess their biomass, taxonomic composition, and physiological status. Typical

bacterial FAs used as trophic markers include 16:1ω7, 18:1ω7 and unique FAs (not present in eukaryotes) such as odd and branched-chain FAs (Kharlamenko et al., 1995; Desvilettes et al., 1997). Microalgae which live in the stream and could potentially colonize leaf detritus are generally high in omega-3 PUFAs (diatoms being especially rich in 20:5ω3) and material with high algal content has a high (>1) ratio of the sum of ω3 to ω6 FAs ($\Sigma\omega$3/$\Sigma\omega$6) (Pollero et al., 1981; Desvilettes et al., 1994).

Although never tested directly, it is reasonable to expect that detritus and associated microorganisms would have different FA profiles under different abiotic and biotic conditions, particularly if different bacterial and fungal species are present (Suberkropp, 1984) or there are changes in metabolic requirements of the microbial community. Biotic interactions within leaf packs in streams may further affect the FA profiles of leaf litter. Both positive (fertilizing) and negative (grazing) effects of invertebrate consumers on leaf detritus are possible. No data of this sort yet exist for streams, although Hunter et al. (2003) demonstrated that certain components of litter chemistry (e.g., nitrogen) were affected by macroinvertebrate exclusion during decomposition in a terrestrial environment. Further efforts are needed to consider how such biotic interactions may affect the quality of leaf detritus in streams. In this study, our objectives were to (1) investigate the FA profiles of leaf detritus from riparian trees and quantify their dynamics during decomposition and (2) assess how these FA dynamics are affected by abiotic (season) and biotic (macroinvertebrate exclusion) factors. Our main hypothesis is that detritus will increase in FA quality (more total FAs and more polyunsaturated FAs) with time, and that quantities of certain FAs typical of fungi and bacteria will increase during decomposition.

Materials and methods

Field procedures

Study area

The study was conducted in a 50-m reach of the Muscoot River, a third-order forested stream that is part of the Croton River watershed in southeastern New York State (N 41° 16.453′; W 73° 44.773′). The streambed consists primarily of boulders (>256 mm, 60%) and cobbles (>64–256 mm, 25%), with smaller amounts of gravel (>2–64 mm, 2%) and sand (0.06–2 mm, 10%). Woody debris typically makes up <5% of submersed substrata. During typical baseflow conditions (average of three dates), stream width = 7.8 m, mean depth = 31 cm, specific conductance = 364 µS/cm, pH = 8.0, NH_4^+ = 100 µg N/l, $NO_2^- + NO_3^-$ = 1000 µg N/l, Si = 1.5 mg/l, total dissolved P (TDP) = 80 µg/l, and Ca = 20 mg/l. Land use is primarily undisturbed mixed-deciduous forest (*Quercus rubra*, *Acer rubrum*, *Platanus occidentalis*, *Tsuga canadensis*). The stream is shaded from May to October (mean canopy cover = 75%), leaf fall is from late October to November, and maximum light penetration (80–90% open sky) occurs from late November to early April. A previous study has examined fatty content of consumers and periphyton in this stream (Torres-Ruiz et al., 2007).

Spring experiment

Autumn-shed leaf litter of the three most abundant riparian tree species (*A. rubrum*, *Q. rubra*, and *P. occidentalis*) was collected from the banks of the Muscoot on 9 May 2004 (shed in autumn 2003) and allowed to air dry for 3 days at room temperature. Species were combined in proportions similar to their presence in the riparian zone (60% maple, 20% oak, 20% sycamore by mass), and placed in each bag (after Boulton & Boon, 1991). A total of 3 g of leaf material was placed in each of 50, 5 mm mesh nylon bags (20 cm × 20 cm), tagged with numbers from 1 to 50. The bags allowed most macroinvertebrate collectors and shredders access to the leaf packs. Five identical groups of leaves were set aside as time-zero samples which were processed within 24 h (see below). Bags were placed in the stream on 13 May 2004 by staking them to the streambed with metal stakes (after Sponseller & Benfield, 2001). Groups of ten bags were placed in five different reaches in locations where an obvious accumulation of leaf litter was observed. Caution was taken not to place bags under sediments. Every 30 days (June, July, Aug, Sept), five bags were selected using a random numbers table, collected, placed in plastic bags, and brought back to the lab on ice where they were processed in approximately 2 h.

Autumn experiment

Freshly fallen leaf litter (shed and collected in autumn 2004) was collected from the same site banks on 10 November 2004 and brought back to the lab to air dry for 3 days at room temperature. Bags were prepared as for the spring with 75 5 mm mesh nylon bags and twenty 250 μm mesh nitex bags. The 250-μm mesh bags were added to the design to exclude macroinvertebrate colonization of leaf litter. After separating five replicates for time-zero analysis (processed within 24 h), all other bags were placed in the stream on 14 November as described above. All large and small mesh bags were grouped together and from the group, 10–20 bags were placed randomly in each one of the reaches selected. As in the spring experiment, five of the 5-mm mesh bags were removed randomly in 30-day intervals on December (2004), January, February, and March (2005). In addition, five 250-μm mesh bags were also removed on both December 2004 and February 2005. All bags were brought back to the lab and processed as above.

Laboratory processing

Leaf litter was briefly rinsed in a beaker with tap water (non-chlorinated well water) and then gently blended with Milli-Q^{TM} deionized water to remove silt but not the microbial film (observed by microscopy). Next, the material was blot-dried on Kim-Wipes^{TM} and two fractions were separated: (1) for FA analysis (placed under N_2 in a centrifuge tube at −20°C) and (2) for dry mass (weighed, placed in a labeled 50 mm aluminum dish). Concentration of FAs was determined by using the dry mass of a subsample and the amount of FA in the other. It was not the intent of this study to determine rates of mass loss, which have been well documented. In addition, all leaf litter in the bags remaining over time was needed to provide a sufficient quality if material for FA analyses, which require fresh (non-dried) material. Subsamples separated for dry mass (including time-zero samples) were dried for at least 1 week (to constant mass) at 80°C and re-weighed using a Mettler Toledo AG204 analytical balance (nearest 0.1 mg). Samples for FA analysis were weighed, homogenized, and extracted using a modified version of Parrish (1999; details in Torres-Ruiz et al., 2007). Briefly, lipids were extracted in 2:1 chloroform:methanol, methylated using BF_3, resuspended in hexane and concentrated under N_2. Fatty acid methyl esters (FAMEs) were separated and quantified using a Hewlett-Packard 5890 gas chromatograph (Wilmington, DE) fitted with a Supelco (Bellfonte, Pennsylvania) Omegawax 320 capillary column (chromatography details in Torres-Ruiz et al., 2007). Individual FAs were identified by comparing retention times with certified standard FA mixtures (e.g., Supelco^{TM} 37 Component FAME mix, Menhaden Oil: PUFA-3 and Bacterial Acid Methyl Esters [BAME] Mix). Our use of three specific indicator FAs as fungal (18:2ω6, 18:1ω9) and bacterial (16:1ω7) markers, despite their abundance in other living organisms was based on four lines of reasoning: (1) measurements were made on dead leaves, which have lost most of these FAs prior to leaf fall; (2) microscopic examination and chlorophyll-*a* analyses of subsamples of detritus demonstrated that green algae and diatoms did not colonize the material, (3) an conscientious effort was made to remove most (if not all) animals from the samples before analysis, and (4) no other diatom markers (e.g., 20:5ω3) were detected in our sample. We suggest that these are important distinctions between measurements made on planktonic seston and benthic leaf litter.

Data analysis

Individual FA concentrations per unit mass were calculated using a regression curve from known weights of standards versus areas obtained in the chromatograms. Calibration curves were linear and highly significant, but slopes differed slightly depending on chain length, number of double bonds and concentration range (observed previously by Ahlgren et al., 1992). A mean slope was therefore chosen to calculate absolute concentrations of total FAs (concentrations in this study were similar to those found by Mills et al., 2001, who used 17:0 as an internal standard). Total FAs, polyunsaturated FAs, and microbial FAs were calculated on both an absolute (mg/g dry mass) and relative (% of total FAs) basis. To assess whether the FA composition of leaf litter changed during decomposition, a one-way ANOVA (Factor = time) was used to independently compare polyunsaturated and total FA percent/content (% and mg/g), as well as the ratio of $\Sigma\omega3/\Sigma\omega6$, bacterial, fungal, and detrital FAs between time-zero and each 30-day interval for both experiments (Systat

v. 11). A two-way ANOVA (Factors = time, invertebrate exclusion) was used to assess decomposition and macroinvertebrate effects on FAs in the autumn experiment, and their interaction. Tukey's post-hoc tests were performed to test for individual differences between time-zero, 30, 60, 90, and 120 days in both the spring and autumn experiments in cases where a general ANOVA identified a general time effect ($P \leq 0.05$). In the autumn experiment Tukey's test was performed within each factor to identify specific time and invertebrate effects. All tests were performed with an a priori alpha level of $P \leq 0.05$ (Systat v. 11). Data were tested for normality and homogeneity of variance assumptions and transformed where required. Percent data were transformed to arcsine, and concentrations to square root (Sokal & Rohlf, 1995). Reported values represent untransformed means.

Results

In both spring and autumn experiments we observed an obvious decline in the quantity of leaf litter in bags over time, which has been often shown for maple, oak and sycamore leaves in temperate streams of eastern US (Sponseller & Benfield, 2001; Kominoski et al., 2009). In this study, no litter remained in most bags after 120 days and this marked the end of the study.

Both spring and autumn experiments revealed strong and significant effects of time on FA composition of leaf litter in the Muscoot River. Initial concentration of total FAs per unit dry mass was significantly greater in the autumn litter (6.9 mg/g) than in spring-collected litter (5.2 mg/g; $F = 6.3$; $P = 0.03$). Total FA concentrations in litter placed in the stream declined significantly over 120 days in both spring (62%; $F = 10.9$; $P < 0.001$) and autumn (56%; $F = 19.4$; $P = 0.0001$) experiments (Fig. 1). Field observations indicated that smaller mesh bags (which excluded macroinvertebrates) remained intact and had no sign of chewing or holes. In contrast, numerous shredders such as isopods, case-building caddisfly larvae, cranefly larvae, and oligochaetes were observed in the large mesh bags. Nonetheless, total FA content of litter placed in bags that excluded macroinvertebrates was not significantly different from that in bags that allowed access (Fig. 1).

The most abundant PUFAs measured in leaf detritus were 18:3ω3 and 18:2ω6. Quantities of long-chain PUFAs such as 20:4ω6 and 20:5ω3 were either undetectable or very low (Fig. 2). Initial 18:2ω6 values (mg/g and %) were significantly greater in the spring litter than in the autumn litter (ANOVA $F = 670$, $P < 0.0001$). Leaf detritus

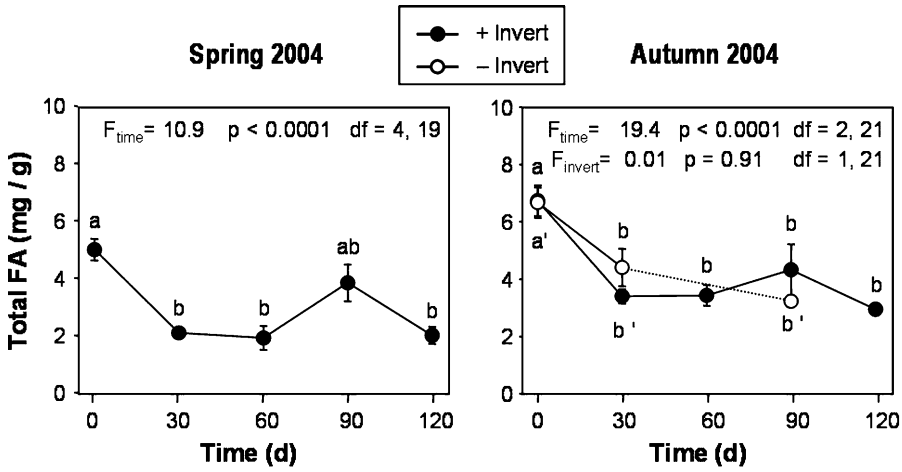

Fig. 1 Temporal changes in concentrations of total FAs (mg/g dry mass) in deciduous leaf litter in the Muscoot River in spring and autumn of 2004 (values are means ± 1 SE; *symbols with different letters* are significantly different [$P < 0.05$] from each other within each experiment; in the autumn, *letters with prime symbol* [e.g., a′] indicate differences within the invertebrate treatment; *closed circles* 5-mm mesh bags that allowed macroinvertebrate colonization; *open circles* 250-μm mesh bags that prevented macroinvertebrate colonization)

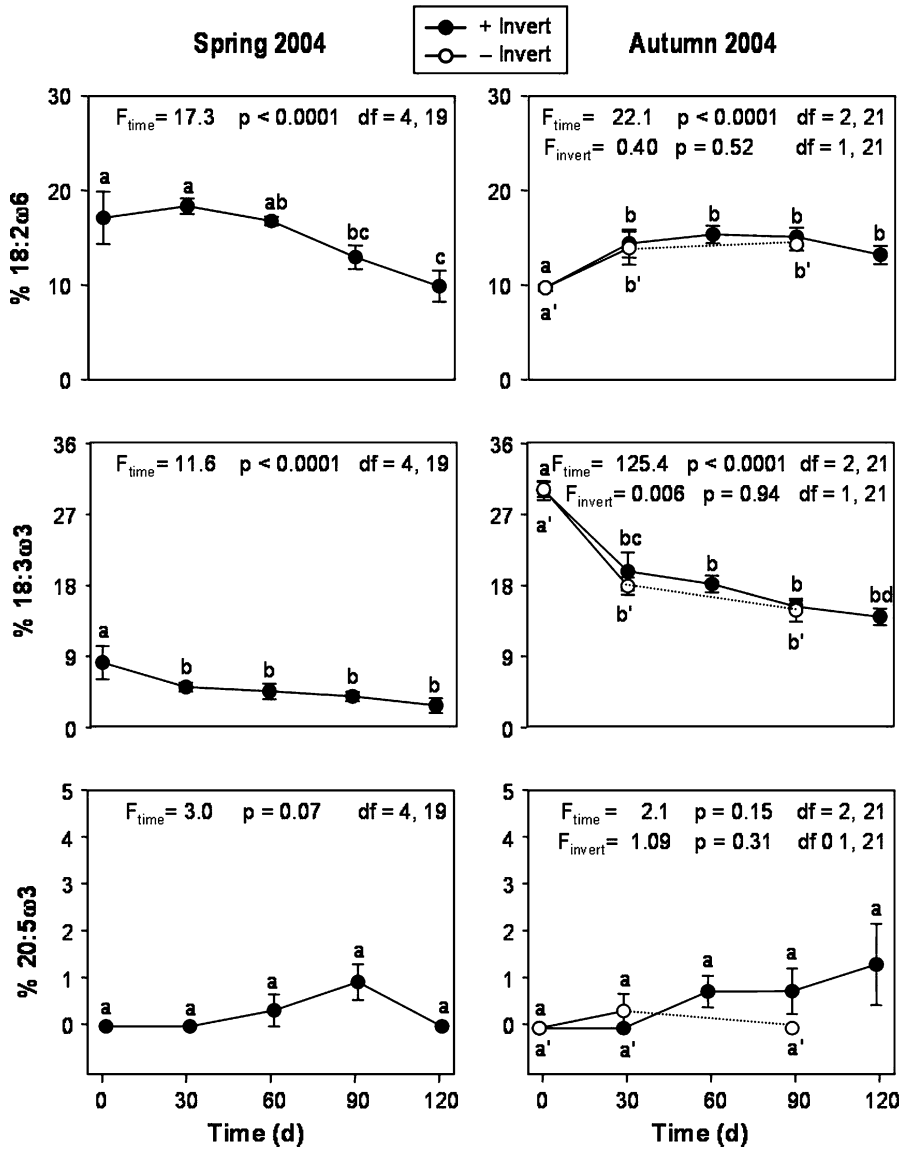

Fig. 2 Temporal changes in percent PUFAs (18:2ω6, 18:3ω3, and 20:5ω3) in deciduous leaf litter in the Muscoot River in the spring and autumn of 2004 (values are means $\pm$ 1 SE; *symbols with different letters* are significantly different [$P < 0.05$] from each other within each experiment; in the autumn, *letters with prime symbol* [e.g., a′] indicate differences within the invertebrate treatment; *closed circles* 5-mm mesh bags that allowed macroinvertebrate colonization; *open circles* 250-µm mesh bags that prevented macroinvertebrate colonization)

collected in spring continually declined in 18:2ω6 content to roughly one-fourth that of initial concentrations (0.9–0.2 mg/g) within 120 days (Table 1) whereas the percent 18:2ω6 in autumn litter increased significantly over time and remained significantly greater than levels measured at time-zero (9.7%, Fig. 2). However, absolute concentrations of this FA also decreased after 30 days (0.7–0.4 mg/g; Table 1). In contrast, concentrations and percentages of 18:3ω3 were two- to three-times greater in litter from the autumn than in spring (9.9%, 0.5 mg/g in spring; 30%, 2 mg/g in autumn), but in both experiments this PUFA significantly declined with time (Fig. 2; Table 1). Both spring and autumn detritus initially had non-detectable levels of 20:5ω3. This PUFA increased slightly with time, although trends were non-significant (Fig. 2; Table 1). In the spring, the ratio of the sum of ω3 to ω6 FAs (aquatic matter marker), $\Sigma\omega3/\Sigma\omega6$, was always lower than 1.0; in autumn, values were $\approx$2 at time-zero and halved to $\approx$1 after 30 days and thereafter (Fig. 3). Changes in PUFAs and $\Sigma\omega3/\Sigma\omega6$ with time were not significantly affected by macroinvertebrate colonization (Figs. 2, 3).

Saturated and monounsaturated FAs were more abundant in leaf detritus than were polyunsaturated

Table 1 Temporal changes (days spent in the stream) in mean concentrations ($n = 5$) of FAs (mg/g dry mass) in leaf litter in the Muscoot River

Day	Spring	Autumn	
Mesh:	5 mm	5 mm	250 μm
18:2ω6			
0	0.88 (0.01)	0.67 (0.05)	0.65 (0.05)
30	0.42 (0.00)	0.52 (0.06)	0.64 (0.18)
60	0.35 (0.07)	0.55 (0.09)	NS
90	0.51 (0.09)	0.68 (0.18)	0.49 (0.01)
120	0.21 (0.07)	0.41 (0.06)	NS
18:3ω3			
0	0.51 (0.00)	2.05 (0.22)	2.03 (0.22)
30	0.16 (0.00)	0.70 (0.02)	0.81 (0.07)
60	0.14 (0.05)	0.64 (0.07)	NS
90	0.24 (0.04)	0.67 (0.11)	0.49 (0.02)
120	0.11 (0.03)	0.42 (0.01)	NS
20:5ω3			
0	<0.01	<0.01	<0.01
30	<0.01	<0.01	0.02 (0.02)
60	0.01 (0.01)	0.03 (0.01)	NS
90	0.04 (0.01)	0.04 (0.02)	<0.01
120	<0.01	0.04 (0.03)	NS
16:1ω7			
0	0.01 (0.01)	<0.01	<0.01
30	0.14 (0.00)	0.10 (0.01)	0.18 (0.01)
60	0.26 (0.02)	0.18 (0.02)	NS
90	0.27 (0.02)	0.29 (0.08)	0.23 (0.02)
120	0.14 (0.01)	0.29 (0.05)	NS
18:1ω7			
0	0.08 (0.00)	0.05 (0.01)	0.04 (0.01)
30	0.10 (0.00)	0.08 (0.00)	0.14 (0.01)
60	0.11 (0.01)	0.12 (0.01)	NS
90	0.25 (0.03)	0.18 (0.06)	0.14 (0.01)
120	0.12 (0.02)	0.12 (0.01)	NS
O + B			
0	<0.01	0.08 (0.00)	0.07 (0.00)
30	0.01 (0.00)	<0.01	<0.01
60	0.02 (0.00)	0.01 (0.00)	NS
90	0.18 (0.01)	0.04 (0.00)	0.01 (0.00)
120	0.13 (0.00)	0.03 (0.00)	NS
18:1ω9			
0	0.61 (0.07)	0.33 (0.03)	0.32 (0.04)
30	0.28 (0.03)	0.47 (0.07)	0.63 (0.20)
60	0.21 (0.03)	0.52 (0.09)	NS
90	0.43 (0.06)	0.66 (0.14)	0.44 (0.02)
120	0.19 (0.03)	0.38 (0.06)	NS
LCSAFA			
0	0.41 (0.00)	0.32 (0.00)	0.33 (0.00)
30	0.17 (0.00)	0.22 (0.00)	0.29 (0.00)
60	0.09 (0.01)	0.23 (0.00)	NS
90	0.28 (0.01)	0.36 (0.01)	0.29 (0.00)

Columns represent different treatments (S 5 mm = allowed for invertebrate colonization, started May 2004; A 5 mm = allowed for invertebrate colonization, started November 2004; A 250 μm = excluded invertebrate colonization, started November 2004; NS = not sampled). Values in parenthesis are 1 SE of means (SE was not calculated for means <0.01; SE <0.01 are shown as 0.00). O + B = Odd plus BAME FAs; LCSAFA: sum of all long-chain saturated FAs (20:0, 22:0, and 24:0)

FAs. The principal FAs were 16:0 and 18:0 (ubiquitous in biological materials, data not shown), 16:1ω7 (bacterial marker), 18:1ω9 (fungal marker), 18:1ω7 (bacterial marker), and odd chain and BAME FAs, which are characteristic of prokaryotes. Also abundant were long-chain saturated FAs (20:0, 22:0, and 24:0). Quantities of potential bacterial markers 16:1ω7, 18:1ω7 and odd + BAME increased with time in both the spring and autumn experiments (percent and mg/g) (Fig. 4; Table 1). Initially, detritus had negligible amounts of 16:1ω7, but, in spring, these increased significantly to a peak of 12.6% (0.26 mg/g) by 60 days and then declined to 30-day levels (Fig. 4; Table 1). In the autumn, quantities of 16:1ω7 increased (% and mg/g) with time to ≈9.2% (0.3 mg/g) by 120 days (Fig. 4; Table 1). Amounts of 18:1ω7 were initially low in both seasons, but increased to reach ≈6% (0.25 mg/g) in the spring and ≈3% (0.18 mg/g) in the autumn after 90 days (Fig. 4; Table 1). The sum of odd chain and BAME FAs was initially very low in spring detritus, but increased tenfold (6%; 0.18 mg/g) after 120 days (Fig. 4; Table 1). In the autumn this marker initially decreased and remained at low levels (Fig. 4; Table 1). Changes in these microbial FAs were not significantly different in bags with or without macroinvertebrate colonization (Fig. 4).

Quantities of the fungal marker 18:1ω9 initially were twofold greater in spring leaf litter than in the autumn and their temporal trends in the two experiments were opposite (Fig. 5; Table 1). In the spring

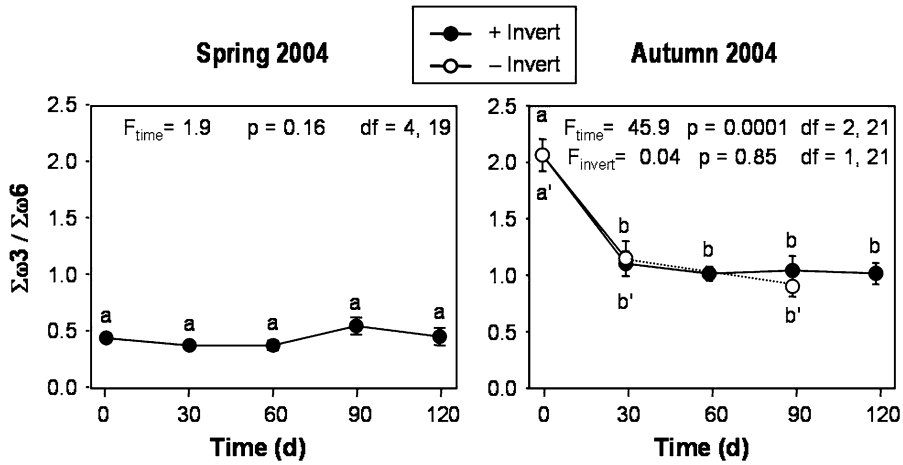

Fig. 3 Temporal changes in the ratios of total omega-3 FAs divided by total omega-6 FAs in deciduous leaf litter in the Muscoot River in spring and autumn of 2004 (values are means ± 1 SE; *symbols with different letters* are significantly different [$P < 0.05$] from each other within each experiment; in the autumn, *letters with prime symbol* [e.g., a′] indicate differences within the invertebrate treatment; *closed circles* 5-mm mesh bags that allowed macroinvertebrate colonization; *open circles* 250-μm mesh bags that prevented macroinvertebrate colonization)

study, levels of this FA decreased by about 9% (0.19 mg/g) after 120 days, while autumn levels increased significantly by about 12% (0.6 mg/g) within 90 days. The percentage of long-chain saturated FAs (LCSAFA; angiosperm detritus indicator; Napolitano, 1999) in autumn increased with time, although concentrations remained fairly stable (≈0.3 mg/g) (Fig. 5; Table 1). There were no significant changes in the percentage of this marker during the spring but concentrations declined significantly (Fig. 5; Table 1).

Discussion

The FA content of leaf detritus that entered the Muscoot River significantly changed during decomposition in both the spring and autumn of 2004. The general hypothesis that detritus will increase in FA quality (more total FAs, more polyunsaturated, essential FAs) during this process was, however, not supported by our data.

It is well established that microbial conditioning alters and in many ways improves leaf litter palatability for consumers (Gessner et al., 1999). Microbes digest, assimilate, and transform many of the complex compounds present in leaf litter to produce their own biomass. But to date, only certain elemental nutrients such as nitrogen have been observed to increase in litter during decomposition in streams (Suberkropp et al., 1976). In general, all other compounds, including carbohydrates and proteins, as well as total mass, have consistently been shown to decline during breakdown (Allan, 1995). Also, because certain parts of leaves are more difficult to digest and assimilate (e.g., lignin), their importance can increase, relative to total mass or other compounds (Suberkropp et al., 1976). Readily assimilated compounds such as carbohydrates and lipids are lost rapidly due to microbial processing (Suberkropp et al., 1976; Bridson, 1985), as well as by physical and chemical processes such as abrasion and oxidation (Allan, 1995). This study agrees with this general finding, since concentrations of total FAs (per gram dry mass) significantly decreased in leaves with time regardless of time of year when the experiment started (Fig. 1). Hence, even though microbes assimilate some FAs and perhaps synthesize other FAs from compounds obtained from leaves, their own metabolic demands apparently prevent them from increasing the FA quality of decomposing leaf litter. Considering the importance of certain essential FAs for aquatic and stream invertebrates (Brett & Mueller-Navarra, 1997; Torres-Ruiz et al., 2007), this trend refutes, at least in part, earlier arguments that microbial colonization enhances the nutritional quality of leaf litter in streams. Indeed, our data (Fig. 1; Table 1) demonstrate that concentrations of FAs

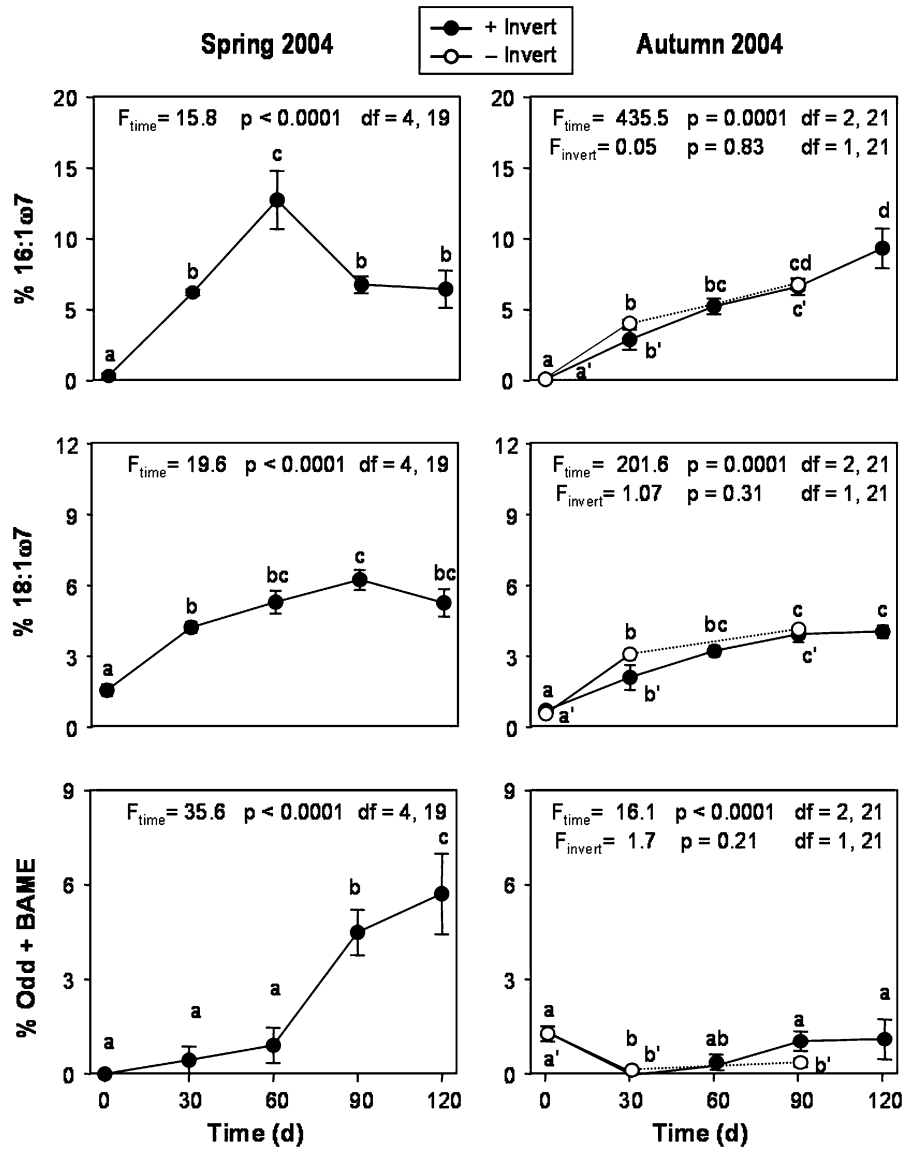

Fig. 4 Temporal changes in percentages of bacterial FAs (16:1ω7, 18:1ω7, and odd + BAME) in deciduous leaf litter in the Muscoot River in spring and autumn of 2004 (values are means ± 1 SE; *symbols with different letters* are significantly different [$P < 0.05$] from each other within each experiment; in the autumn, *letters with prime symbol* (e.g., a′) indicate differences within the invertebrate treatment; *closed circles* 5-mm mesh bags that allowed macroinvertebrate colonization; *open circles* 250-µm mesh bags that prevented macroinvertebrate colonization)

declined at a rate faster than did bulk leaf matter. This is in agreement with the one other study that examined FA dynamics in leaf litter in a blackwater stream in southeastern USA (Mills et al., 2001).

Quantities of total FAs in leaf litter from both spring and autumn decreased by half with time in the Muscoot and, although there was a slight increase after 90 days in the spring experiment (perhaps peak microbial activity), values never exceeded initial levels. The low amounts of FAs in leaf litter were likely furthered by oxidation and resorption of FAs by the tree during senescence prior to leaf fall (Merzlyak & Hendry, 1993). While our study specifically examined autumn-shed leaves, the role of resorption is supported by a comparison of green leaves that had fallen during a summer storm with autumn-shed litter used in our experiments. Green leaf litter had total FA concentrations of ≈12 mg/g, which is 40% greater than initial levels in autumn litter and 65% greater than aged autumn-shed litter collected in spring (Fig. 1; unpublished data).

Macroinvertebrates can affect the chemistry of litter during decomposition (Hunter et al., 2003), but this study observed no macroinvertebrate effect on

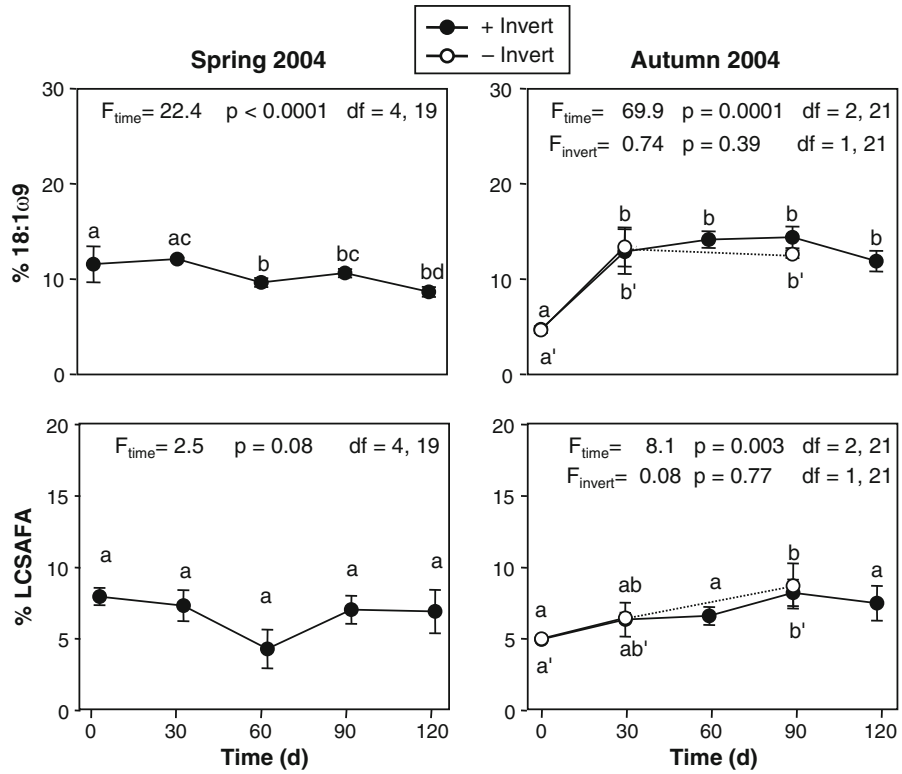

Fig. 5 Temporal changes in percentages of fungal (18:1ω9), and detrital FAs (LCSAFA) in deciduous leaf litter in the Muscoot River in spring and autumn of 2004 (values are means ± 1 SE; *symbols with different letters* are significantly different [$P < 0.05$] from each other within each experiment; in the autumn, *letters with prime symbol* [e.g., a′] indicate differences within the invertebrate treatment; *LCSAFA* sum of all long-chain saturated FAs [20:0, 22:0, and 24:0]; *closed circles* 5-mm mesh bags that allowed macroinvertebrate colonization; *open circles* 250-µm mesh bags that prevented macroinvertebrate colonization)

the FA composition of litter. This is surprising, because the larger (5 mm) mesh bags harbored a variety of detritivores (isopods, case-building caddisflies, craneflies, oligochaetes), while none were observed in 250-µm mesh bags. Macroinvertebrates from these groups have been shown to consume maple and oak leaves in other woodland streams (Eggert & Wallace, 2003). Suberkropp (2003) has stated that although macroinvertebrates feeding on leaves may reduce fungal activity (perhaps through ingestion), most studies show that fungal community structure is not affected. Since we did not measure total mass loss in leaf bags (although quantities obviously declined), it is possible that macroinvertebrates fed on leaf detritus unselectively. But if they had been preferentially feeding on fungi (and other microbes), as some investigators have proposed (Suberkropp, 2003), total FA concentrations would have decreased more steeply in 5-mm mesh bags, which allowed macroinvertebrate access. Our data suggest that this was not the case as none of the measured fungal/microbial FAs were affected by macroinvertebrate inclusion or exclusion. These treatments did not affect FA composition or concentrations during decomposition, so perhaps other factors (e.g., leaching, microbial activity, oxidation) may have greater effects on this process.

Only three essential PUFAs were detected in measurable quantities in leaf detritus: 18:2ω6, 18:3ω3, and 20:5ω3. This contrasts with much greater total quantities and wider variety of PUFAs in algal material in the same stream (Torres-Ruiz et al., 2007). Quantities of these three changed significantly over time in the spring and autumn experiments, with the trends and magnitudes depending on the specific FA and time of year. Another

essential PUFA, arachidonic acid (20:4ω6), was always undetectable. It is known that 18:2ω6 and 18:3ω3 are present in live, green leaves, as they are a prominent part of the photosynthetic membranes of chloroplasts (Napolitano, 1999; Mills et al., 2001), but their temporal patterns during decomposition were largely negative. In addition, 18:2ω6 can be a major component of fungal lipids (Stahl & Klug, 1996; Ruess et al., 2002). The present data indicate that recently abscised (autumn-shed) leaves in this forest have an 18:2ω6 content of about 10% of total FAs (0.67 mg/g) (Fig. 2; Table 1). Leaves that had been decomposing on the adjacent soil since the previous autumn (collected in May 2004) had 30% greater 18:2ω6 concentrations (Fig. 2; Table 1) than recently abscised autumn leaves. These results suggest greater fungal growth and slower depletion of this FA in instances where leaves remain on the soil for some period before entering the stream. However, this greater supply in the spring litter was depleted within 60 days of leaching and decomposition in the stream (percent and absolute concentrations of 18:2ω6, Fig. 2; Table 1). This trend suggests that 18:2ω6 declined more rapidly than all other FAs, and more rapidly than mass loss. In contrast, the percent 18:2ω6 significantly increased in autumn leaves after stream breakdown started (Fig. 2), suggesting that in autumn, this FA was depleted at rates slower than other FAs, possibly due to more aquatic fungal activity during this season. Another piece of evidence is provided by 18:1ω9, which is also a prominent fungal lipid (Stahl & Klug, 1996; Ruess et al., 2002). In this study, this FA increased significantly over time (percentage and concentration) during autumn litter decomposition, with levels as high as 15% of total FAs and 0.6 mg/g dry mass (Fig. 5; Table 1). These results collectively suggest greater fungal activity on autumn-shed than spring detritus, at least with respect to their FA composition. This is not surprising considering fungal growth rates, and biomasses are typically greatest in the autumn (Suberkropp, 1997).

Rapid rates of decline in the PUFA α-linolenic acid (18:3ω3) suggest that it was preferentially depleted over other FAs in both the spring and autumn. This is evidenced by a decrease in percentage (relative to other FAs) as well as concentration (mass basis) over time (Fig. 2; Table 1). This was further reflected in a sharp decrease in the $\Sigma\omega3/\Sigma\omega6$ ratio (Fig. 3) in autumn. Our data agree with observations by Mills et al. (2001) of preferential degradation of unsaturated FAs over other FAs in leaf litter (although different tree species). Microbial processing as well as oxidation reactions to which PUFAs are particularly susceptible could account for this loss. The fact that spring leaf litter initially had lower 18:3ω3 content (Fig. 2; Table 1) likely means that soil decomposition also affected this FA in litter.

Another PUFA, eicosapentaenoic acid (20:5ω3), is not a major component of terrestrial leaves, but is commonly abundant in certain freshwater microalgae, especially diatoms (Dunstan et al., 1994; Napolitano, 1999). In this study, initial levels of this FA were negligible in both spring and autumn litter. Temporal effects on both sources of litter were non-significant and 20:5ω3 remained low (Table 1). This was correlated with low chlorophyll-a values observed in the fall (unpublished data) and also by the decreasing and low $\Sigma\omega3/\Sigma\omega6$ ratio. All together these data suggest negligible microalgal growth on decomposing litter.

The activity of bacteria in this study was suggested by the presence of certain bacteria FAs in detritus (16:1ω7, 18:1ω7; sum of odd chain and BAME FAs) which have been applied by others as bacterial markers in other ecosystems (Kharlamenko et al., 1995; Desvilettes et al., 1997). All of these markers were detected in spring and autumn detritus in this study and their content increased with time (Fig. 4). Levels of these bacterial markers in leaves were initially almost undetectable in both experiments, which suggest that soil decomposition had not promoted any substantial bacterial biomass. The 16:1ω7, 18:1ω7, and especially "odd + BAME" content in spring detritus was greater than in autumn detritus through most of this study (Fig. 4; Table 1), possibly suggesting more bacterial activity in the spring.

It has been suggested that fungal activity on leaf litter and other forms of detritus may inhibit bacterial growth through production of antibacterial agents and direct competition (Gulis & Suberkropp, 2003). In two Alabama streams, fungal biomass decreased as bacterial biomass increased (Weyers & Suberkropp, 1996), but the stream with greater nutrients had bacterial productivity similar to that of fungi. In other temperate streams, fungal biomass has been observed to be least in the spring and greatest in autumn (Suberkropp, 1997; Methvin & Suberkropp, 2003).

These findings may have some bearing on this study. Indirect evidence from the FA presented in this study suggests that bacterial activity was greater on detritus in spring than in autumn, which presumably had more fungal activity. In the spring experiment, terrestrial fungi colonizing leaves that had fallen the previous autumn might have been inhibited by conditions in the water, perhaps allowing bacteria to dominate the decomposition process. This study observed a rapid loss of fungal markers (18:2ω6 and 18:1ω9) over time and an increase in bacterial FAs (e.g., 16:1ω7, 18:1ω7). In autumn, a clear fungal signature was observed during most of the experiment (greater 18:2ω6 and 18:1ω9), but declined by the end of the experiment, at a time when bacterial activity was apparently increasing.

Further measurements of FA composition in litter could provide better insights into the nutrition of allochthonous matter and the relative importance of fungi and bacteria during decomposition. Currently, the methods used to investigate fungal and bacterial productivity are the incorporation of ^{14}C-acetate into ergosterol and ^{3}H-leucine into proteins, respectively, but these methods are not entirely comparable (Weyers & Suberkropp, 1996). Recently, the use of ^{13}C-acetate to label specific phospholipid FAs (e.g., 18:1ω9 in fungi and 16:1ω7 in bacteria) has been applied to assess relative importance of bacteria and fungi in ryegrass straw decomposition (McMahon et al., 2005). Data from this study suggest that certain other FAs (18:1ω7, odd FAs) may be specific enough to help in future techniques to quantify specific players in leaf decomposition processes in streams.

Long-chain saturated FAs have been used as markers of terrestrial leaves in other freshwater systems (Napolitano, 1999). Many of these FAs are part of cuticular waxes (Mills et al., 2001). This study confirmed that these FAs were one component of the detrital lipids of leaf litter in the Muscoot River (Fig. 5). Together with FAs characteristic of microbes, these FAs could be used as markers of leaf detritus in streams.

Past feeding experiments have shown that stream macroinvertebrates preferentially consume litter that is microbially colonized over sterile detritus (Suberkropp, 2003). Cargill et al. (1985a, b) suggested that lipids in colonized detritus were responsible for this preference. However, our study has demonstrated that total FA content of leaves actually decreased with time in the Muscoot River, and that essential PUFAs, such as 18:3ω3, were preferentially degraded and practically lost after the initial 30 days in this stream. Our data suggest that the observed preference for colonized detritus by macroinvertebrates may be due to other factors such as an increase in nutrients (e.g., phosphorus or nitrogen) and/or protein or carbohydrate quality, rather than FAs. This study is the first to investigate changes in FA profile in decomposing autumn-shed leaves in a forested stream. The method holds promise in helping understand decomposition processes in streams, the nutritional dynamics of decomposing leaf litter, and how fungal and bacterial colonization may contribute to macroinvertebrate production in stream ecosystems.

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