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Leaf Breakdown in a Tropical Stream

key words: invertebrates, bacteria, fungi, yeast, chemical composition.

Abstract

The objectives of this study were to investigate leaf breakdown in two reaches of different magnitudes, one of a 3rd (closed riparian vegetation) order and the other of a 4th (open riparian vegetation) order, in a tropical stream and to assess the colonization of invertebrates and microorganisms during the processing of detritus. We observed that the detritus in a reach of 4th order decomposed 2.4 times faster than the detritus in a reach of 3rd order, in which, we observed that nitrate concentration and water velocity were greater. This study showed that the chemical composition of detritus does not appear to be important in evaluating leaf breakdown. However, it was shown to be important to biological colonization. The invertebrate community appeared not to have been structured by the decomposition process, but instead by the degradative ecological succession process. With regards to biological colonization, we observed that the density of bacteria in the initial stages was more important while fungi appeared more in the intermediate and final stages.

1. Introduction

In streams with well developed riparian vegetation, the study of decomposition of leaf detritus of allochthonous origins is fundamental to understanding energy flow and metabolism maintenance (HENRY *et al.*, 1994; BENFIELD, 1997; WALLACE *et al.*, 1997). Decomposition is characterized by three phases: leaching, conditioning and fragmentation, yet, this sequence should not be considered merely a temporal and successional process, but rather as overlapping and interacting events (GESSNER *et al.*, 1999). The decomposition of leaves is influenced by water characteristics such as the concentration of nutrients (SUBERKROPP and CHAUVET, 1995), temperature (IRONS *et al.*, 1994), water discharge, and pH (WEBSTER and BENFIELD, 1986). But it is also influenced by other factors such as the chemical composition of the detritus (OSTROFSKY, 1997), microorganisms (SUBERKROPP, 1998), and invertebrates (GRAÇA, 2001).

The microbial community on leaf detritus consists, basically, of fungi and bacteria. In terms of biomass bacteria on leaves are less important than fungi (GRAÇA, 2001). In general, bacteria colonize rapidly during the initial stages of decomposition acting upon easily assimilated molecules (PETERSEN *et al.*, 1989; TANAKA, 1991; JUGNIA *et al.*, 2000). Special attention has been given to fungi, evaluating their role in decomposition and their ability to increase the nutritional value of a substrate (quality of detritus), due to their capacity to

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metabolize molecules that do not easily decompose such as cellulose and lignin (ABELHO and GRAÇA, 1996; PEREIRA *et al.*, 1998; CANHOTO and GRAÇA, 1999; HAAPALA *et al.*, 2001).

Invertebrates are important in decomposition, mainly in accelerating the process, by fragmenting the detritus into small pieces, thereby increasing the surface area available for microbial action (GRAÇA, 2001). However, the role of these organisms has not yet been determined for tropical streams (MATHURIAU and CHAUVET, 2002; DOBSON *et al*., 2003).

Headwater streams are strongly influenced by the drainage basins in which they occur. Another important factor is the amount of light that the canopy of the riparian vegetation blocks out. Shading caused by riparian vegetation results in an insufficient amount of light to power autochthonous primary production, causing this type of primary production to be incapable of supporting existing biological communities in these streams. Therefore, the energy to support these communities is often obtained via allochthonous input of organic matter (WEBSTER and MEYER, 1997; JONES, 1997). It is estimated that up to 99% of the energy that circulates in small rivers has its origins from surrounding forests (FISHER and LIKENS, 1973).

The objectives of this study were: (i) to investigate leaf breakdown in two reaches of different magnitudes, one of a 3rd (closed riparian vegetation) order and the other of a 4th (open riparian vegetation) order in a tropical stream and (ii) to assess the colonization of invertebrates and microorganisms during the processing of detritus.

2. Material and Methods

2.1. Study Area

The Indaiá stream, part of the Rio Doce watershed, is located in the Serra do Cipó National Park, Minas Gerais, Brazil (PARNA Cipó – 19°20′ S and 43°44′ W), in the southern part of the Espinhaço mountain range. The climate in Serra do Cipó is typical for the high altitude tropical with rainy summers and dry winters. Average annual temperatures oscillate between 17.0 and 18.5 °C and the precipitation between 1450 and 1800 mm.

The vegetation in the region is biologically rich with a high rate of endemism (GIULIETTI, 1997). At least three large vegetation formations can be found in the region: tropical savannah (locally called cerrado), rocky fields, and riparian forest.

2.2. Experimental Design

The detritus used in this study, although not identified specifically, was composed of a mixture of recently fallen and commonly encountered senescent leaves from the riparian zone (for an example of vegetation commonly found in this riparian zone see GONÇALVES *et al*., in press). This study was conducted in two reaches of the Indaiá stream: a 3rd order (closed riparian vegetation, 19°16.4′ S – 43°31.2′ W, 1450 m of a.s.l.) and a 4th order (open riparian vegetation, 19°16′ S – 43°10.9′ W, 1380 m of a.s.l.).

Leaf detritus was placed in litter bags (30×30 cm) with a mesh size of 5 mm. The leaves were weighed (approximately 6 ± 0.1 g of dry weight), After 3, 7, 15, 30, 60, 90, 120, 150, 180, 210, 240 and 270 days of immersion, three litter bags were returned to the laboratory for analysis.

In the field, the following water characteristics were measured with a Horiba model U-10 multiprobe: temperature, pH, conductivity, turbidity and dissolved oxygen. The water velocity was also measured with a flow probe (Global Waters Instrumentation Inc.).

After collection, the content of each bag was washed in running water over a 180 μm sieve. The detritus was then dried in a drying oven (60 °C) for 72 h, for later weighing. The breakdown rates were calculated from a negative exponential model (WEBSTER and BENFIELD, 1986). After weighting, the mixed detritus was triturated in order to evaluate the concentrations of Kjeldahl nitrogen (SARRUGE and HAAG, 1974; MALAVOLTA *et al*., 1989), phosphorous (MIYAZAWA *et al*., 1992; MALAVOLTA and NETTO 1989), lignin (VAN SOEST, 1963) and carbohydrates (DERIAZ, 1961; CONN and STUMPF, 1975).

2.3. Microorganisms

For microbiological analysis, one leaf was removed from each sample (litter bag), placed in a sterile bag, and transported to the laboratory on ice to preserve the sample for processing within 8 hours. Each sample was triturated in 2 mL of sterile distilled water using a homogenizer (Polytron PT 2100 Stand Dispersing Equipment) for 30 seconds, previously disinfected with 70% alcohol. To count heterotrophic bacteria 1 mL of the triturated sample was inoculated in triplicate, using the pour plate method, in a standard agar medium – NWRI agar (0.3% soluble peptone; casein; 0.02% K_2HPO_4; 0.005% $MgSO_4$; 0.0001% $FeCl_3$; 1.5% agar). The plates were incubated for 24–48 hours at 25 °C and the results expressed in CFU mL^{-1} (CLESCERI *et al*., 1998). For the isolation of yeasts 0.1 mL of the sample was spread on Ym agar (2% glucose, 1% peptone, 0.3% malt extract, 0.3% yeast extract, 2% agar, 20 mg% chloramphenicol). The plates were incubated for 3–5 days at 25 °C (YARROW, 1998). Colonies were counted and the density of yeasts was estimated by calculating the CFU mL^{-1} of the sample. To evaluate the density of the filamentous fungi 1 mL of the sample was inoculated in triplicate, using the pour plate method, in plates containing Martin medium (0.5% peptone, 1% glucose, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7\ H_2O$, 3.3 mg% Rose bengal, 1.5% agar, pH 5.8) (HORN and DORNER, 1998). The plates were incubated for 3–5 days at 25 °C. Colonies were counted and the results expressed in CFU mL^{-1} of the sample.

2.4. Invertebrates

After washing the leaves over a sieve, the remaining material was preserved in 10% formalin for later identification and quantification under a microscope-stereoscope with 30× magnification. The identified invertebrates were deposited into the Benthic Macroinvertebrate Reference Collection of the Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais. The SHANNON-WIENER diversity index was used to assess invertebrate community according to MAGURRAN (1991). Functional feeding groups were classified according to MERRITT and CUMMINS (1996).

2.5. Statistical Analyses

The abundance data matrix, as well as the substrate chemical composition matrix (ln transformed), was analyzed by canonical correspondence analysis (CCA) (TER BRAAK, 1986) to determine the relative importance of the environmental variables in the invertebrate community structure.

The analysis of variance (one-way ANOVA) was used to test differences in water parameters within the two reaches. The differences between the decay rates in the two studied reaches were tested with an analysis of covariance analysis (ANCOVA). Multi dependent analysis of variance (MANOVA) was used to test the difference between the chemical composition (nitrogen, phosphorous, lignin and carbohydrate-dependent variables) of the leaf detritus, and the functional feeding groups (abundance of each category-dependent variable) that colonized the detritus. Analysis of variance (two-way ANOVA), was used TO test microorganisms (yeast, bacteria and fungi) and invertebrate (density-dependent variable) that colonized the detritus. Time was an independent variable in all analysis from the two different reaches.

3. Results

Results from the water features in the 3rd order (closed riparian vegetation) and 4th order (open riparian vegetation) reach showed that oxygen and depth was significantly greater in the 4th order reach (Table 1). Also of note, the concentrations of PO_4^-, NH_4, NO_3, and velocity values in 4th order reach were higher than in the 3th order.

Leaf breakdown was 2.4 times faster in the 4th order reach, when compared to 3rd order reach (Fig. 1). In the 4th order reach the detritus completely broke down in 240 days ($k = 0.0157$; $R^2 = 0.63$). In the 3rd order reach the detritus breakdown took 270 days after

Table 1. Water parameters (mean and standard deviation) in the 3rd and 4th order reaches of Indaiá stream.

Parameters	3rd order	4th order	F/p
Depth (cm)	15.3 ± 7.8	26.5 ± 7.3	6.6/0.03
Turbidity (NTU)	13.0	20.0	–
Water Velocity (m s^{-1})	3.0 ± 2.3	4.9 ± 3.1	1.2/0.3
Temperature (°C)	18.7 ± 3.1	19.4 ± 4.1	0.1/0.75
Electrical Conductivity (mS cm^{-1})	0.012 ± 0.006	0.010 ± 0.007	0.47/0.5
O_2 (mg L^{-1})	7 ± 0.9	8.7 ± 1.4	7.01/0.02
pH	5.4 ± 2.2	5.4 ± 2.1	0.002/0.9
NH_4^- (μg L^{-1})*	48.00	51.32	–
NO_3 (μg L^{-1})*	5.193	35.00	–
PO_4^- (μg L^{-1})*	3.057	3.256	–

* Data for the tropical Cerrado stream were taken from CALLISTO *et al.* (2004).

incubation, when its remaining weight was 19% of the original dry weight ($k = 0.0065$; $R^2 = 0.85$). These results were significantly different (ANCOVA, $F = 6.17$; $p = 0.0069$).

No variation was observed in the nitrogen concentrations of the detritus of either reach studied (Table 2). The smallest value in the 3rd order reach was 1.15% and the largest was 1.36%. In the 4th order reach the smallest value obtained was 1.03% and the largest 1.47%. Also no variation was observed in the phosphorous concentrations for the detritus from either the 3rd or the 4th order reaches throughout breakdown (0.04 to 0.07%). Lignin concentration was less at the start of breakdown (24.3% in the 3rd order reach and 20.4% in the 4th order reach) and increased, until reaching the maximum of 45.9% in the 3rd order reach (on the 120th day) and 47.7% (on the 180th) day in the 4th order reach. Carbohydrate concentrations displayed an opposite pattern. The maximum observed values were obtained at the start of detritus breakdown (20.11% for both reaches). The minimum value of the 3rd order reach was 9.18% on the 180th and 270th days and 9.59% in the 4th order on the 180th day. Statistical results showed that there was no significant difference in the chemical composition of the detritus between the 3rd and 4th order reaches for each compound studied (ANOVA, $F = 1.94$; $p > 0.05$).

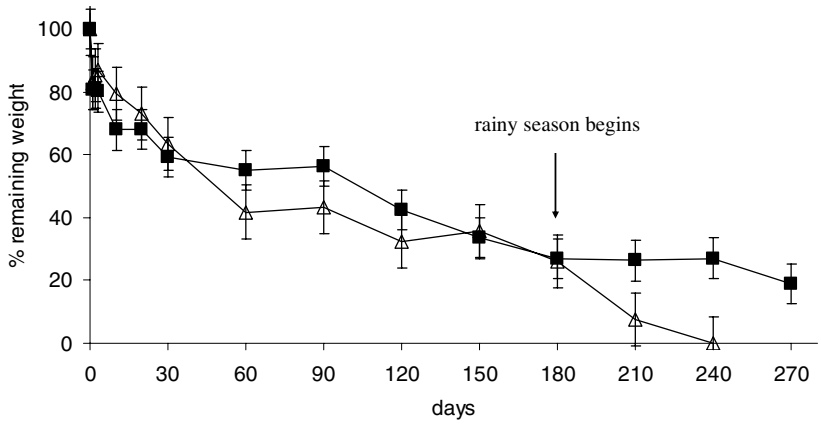

Figure 1. Mass loss of detritus (means of three replicates ± standard error) in the 3rd (square) and 4th (triangle) order reaches of Indaiá stream. (→) indicate the beginning of the rainy season (September).

Table 2. Chemical composition of detritus (% DW) during breakdown in the 3rd and 4th order reaches of Indaiá stream. ND = below limit of detection.

Days	Chemical Composition							
	NITROGEN		PHOSPHOROUS		LIGNIN		CARBOHYDRATES	
	3rd	4th	3rd	4th	3rd	4th	3rd	4th
1	1.33	1.3	0.07	0.06	24.30	20.40	20.11	20.11
2	1.34	1.25	0.06	0.06	21.20	21.70	13.90	19.48
3	1.30	1.12	0.06	0.05	22.70	24.50	21.29	5.31
10	1.28	1.03	0.06	0.04	25.70	27.30	18.27	17.68
20	1.26	1.26	0.05	0.05	31.30	31.20	15.71	18.09
30	1.25	1.17	0.04	0.05	33.20	31.40	12.33	18.90
60	1.30	1.12	0.05	0.04	38.50	28.70	12.51	11.74
90	1.25	1.20	0.04	0.05	43.20	41.20	11.16	10.57
120	1.36	1.19	0.05	0.05	45.90	44.50	11.74	10.57
150	1.15	1.06	0.04	0.05	45.00	47.70	15.88	10.17
180	1.18	1.25	0.05	0.05	44.70	47.20	9.18	9.59
210	1.19	1.47	0.04	0.07	45.60	43.60	11.34	ND
240	1.16		0.04		45.60		10.35	
270	1.15		0.04		42.31		9.18	

Within the invertebrate groups encountered, Chironomidae (Diptera) and Ephemeroptera were present in all the sampling periods, with Chironomidae occurring in the highest densities (Tables 3 and 4), in the 3rd and 4th order reaches (5795 and 5549 ind 100 g^{-1} DW, respectively), although in the 1st and 2nd days Ephemeroptera were dominant. The same was observed for Cladocera on the 210th day; both occurrences were observed in the 3rd order reach. Other groups such as Trichoptera, Oligochaeta, Copepoda, Coleoptera, Odonata, and Hemiptera were also important in both reaches studied. The highest density value for all organisms combined was observed in the 3rd order reach, 10229 ind 100 g^{-1} DW on the 180th day, while the highest density in the 4th reach was 9676 ind 100 g^{-1} DW on the 210th day. The maximum value for the Shannon-Wiener diversity index (H = 0.812), for the 3rd order reach, was obtained on the 60th day. In the 4th order reach the maximum value for this diversity index was found on the 20th day (H = 0.633).

In the incubated detritus from both reaches we observed that the collector invertebrates were the most abundant, followed by the predators, shredders, and scrappers (Tables 3 and 4). The functional feeding groups had a homogenous distribution and their proportions varied little over time (Fig. 2). A significant difference was found between the reaches of 3rd and 4th orders (ANOVA, $F = 18.35$, $p < 0.001$).

The amounts of nitrogen and phosphorous in the detritus were correlated to the invertebrate community at the start of breakdown, while the amount of lignin was correlated to the invertebrates in the more advanced stages of breakdown in both of the reaches studied. The amount of carbohydrate was not correlated to the invertebrates at any point during detritus breakdown (Fig. 3).

The density of heterotrophic bacteria on the detritus, in both reaches, was higher during the following days within each stage: initial stage (1st, 2nd, and 3rd days), intermediate stage (60th and 90th days), and final stage (150th, 180th, 210th, and 240th days) of the decomposition process (Fig. 4A). The highest densities were observed in the initial stages, mainly in the 3rd order reach. The yeasts were frequent on the 2nd, 3rd, 10th, and 90th days, and the highest density was observed in the 4th order reach, on the 90th day (Fig. 4B). The density of filamentous fungi increased beginning the 20th day, in both reaches studied. Highest densities were

Table 3. Invertebrates community that colonized detritus during breakdown in the 3rd order reach of Indaiá stream (mean of three replicates and ± standard deviation of individuals 100 g^{-1} DW) and functional feeding groups (P = predator, FC = Filtering-collector, GC = Gathering-collector, S = Shredder, Sc = Scraper).

Taxa	FFG	days													
		1	2	3	10	20	30	60	90	120	150	180	210	240	270
Annelida															
Hirudinea	P	0 ± 0	0 ± 0	0 ± 0	7 ± 13	7 ± 12	8 ± 14	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Oligochaeta	GC	0 ± 0	6 ± 10	13 ± 22	46 ± 41	663 ± 347	1145 ± 709	728 ± 412	26 ± 25	0 ± 0	131 ± 114	261 ± 193	0 ± 0	0 ± 0	23 ± 40
Arthropoda															
Arachnida															
Acarina	P	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	19 ± 17	0 ± 0	24 ± 22	0 ± 0	148 ± 145	66 ± 58	82 ± 143	49 ± 85
Branchiopoda															
Cladocera	FC/P	6 ± 11	12 ± 22	76 ± 70	208 ± 322	988 ± 433	595 ± 298	451 ± 345	0 ± 0	147 ± 148	94 ± 47	846 ± 666	868 ± 855	49 ± 86	183 ± 81
Ostracoda	FC/P	0 ± 0	0 ± 0	0 ± 0	7 ± 13	0 ± 0	8 ± 14	19 ± 17	19	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Copepoda	CF	6 ± 11	7 ± 11	19 ± 18	29 ± 33	110 ± 21	128 ± 86	443 ± 187	0 ± 0	238 ± 173	217 ± 191	800 ± 664	648 ± 485	52 ± 50	69 ± 119
Insecta															
Coleoptera	Sh/GC/Sc/P	0 ± 0	0 ± 0	6 ± 11	22 ± 38	0 ± 0	67 ± 49	77 ± 46	17 ± 29	53 ± 69	54 ± 63	532 ± 368	721 ± 451	252 ± 182	179 ± 63
Diptera															
Ceratopogonidae	P	0 ± 0	0 ± 0	0 ± 0	0 ± 0	7 ± 13	0 ± 0	52 ± 65	0 ± 0	29 ± 50	0 ± 0	50 ± 43	67 ± 81	0 ± 0	0 ± 0
Chaoboridae	P	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	26 ± 44	0 ± 0	0 ± 0	0 ± 0
Chironomidae	P/GC	38 ± 32	87 ± 19	146 ± 74	1837 ± 1317	1645 ± 314	2364 ± 987	2009 ± 510	571 ± 272	1120 ± 65	2070 ± 1127	5795 ± 4738	723 ± 288	253 ± 84	1608 ± 486
Ephemeroptera	GC	83 ± 59	151 ± 56	76 ± 69	384 ± 330	145 ± 158	241 ± 159	461 ± 249	150 ± 125	818 ± 376	929 ± 797	975 ± 250	192 ± 80	110 ± 115	653 ± 349
Heteroptera	P	0 ± 0	12 ± 22	6 ± 11	0 ± 0	0 ± 0	0 ± 0	10 ± 18	0 ± 0	0 ± 0	13 ± 23	122 ± 150	0 ± 0	0 ± 0	0 ± 0
Hemiptera	P	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	8 ± 14	18 ± 16	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	121 ± 154
Odonata	P	6 ± 11	0 ± 0	0 ± 0	29 ± 50	31 ± 36	8 ± 14	48 ± 15	0 ± 0	0 ± 0	34 ± 31	38 ± 38	0 ± 0	0 ± 0	0 ± 0
Trichoptera	Sh/FC/GC/Sc/P	13 ± 11	0 ± 0	45 ± 30	75 ± 36	98 ± 99	114 ± 79	281 ± 217	201 ± 127	170 ± 90	281 ± 190	585 ± 748	314 ± 196	61 ± 106	44 ± 38
Hidrozoa															
Hydra	P	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	106 ± 62	63 ± 83	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Mollusca															
Gastropoda	Sc	0 ± 0	0 ± 0	0 ± 0	36 ± 63	64 ± 92	103 ± 135	33 ± 58	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Nematoda	P	0 ± 0	0 ± 0	0 ± 0	23 ± 23	65 ± 19	34 ± 13	67 ± 7	17 ± 29	0 ± 0	0 ± 0	51 ± 89	0 ± 0	0 ± 0	0 ± 0
Rotifera	P	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	13 ± 23	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Total Density		152	276	387	2704	3824	4921	4770	999	2600	3836	10229	3599	859	2929
Diversity H′		0.552	0.496	0.716	0.509	0.667	0.679	0.812	0.535	0.634	0.598	0.680	0.787	0.750	0.607

Table 4. Invertebrates community that colonized detritus during breakdown in the 4th order reach of Indaiá stream (mean of three replicates and ± standard deviation of individuals 100 g^{-1} DW) and functional feeding groups (P = predator, FC = Filtering-collector, GC = Gathering-collector, S = Shredder, Sc = Scraper).

Taxa	FFG	days											
		1	2	3	10	20	30	60	90	120	150	180	210
Annelida													
Oligochaeta	GC	0 ± 0	0 ± 0	0 ± 0	12 ± 11	41 ± 70	10 ± 17	191 ± 243	0 ± 0	228 ± 395	260 ± 329	0 ± 0	92 ± 159
Arthropoda													
Arachnida													
Acarina	P	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	36 ± 41	123 ± 141
Branchiopoda													
Cladocera	FC/P	6 ± 11	0 ± 0	0 ± 0	49 ± 55	256 ± 177	277 ± 137	110 ± 65	0 ± 0	23 ± 40	101 ± 133	175 ± 153	92 ± 159
Copepoda	FC	0 ± 0	6 ± 10	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	11 ± 20	0 ± 0	19 ± 33	226 ± 289
Insecta													
Coleoptera	Sh/GC/Sc/P	0 ± 0	0 ± 0	0 ± 0	0 ± 0	7 ± 12	44 ± 12	60 ± 17	0 ± 0	0 ± 0	176 ± 190	540 ± 316	1457 ± 879
Diptera													
Ceratopogonidae	P	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	18 ± 31	0 ± 0	0 ± 0	11 ± 20	13 ± 22	0 ± 0	0 ± 0
Chironomidae	P/GC	777 ± 105	497 ± 287	84 ± 73	1270 ± 632	503 ± 41	939 ± 270	1057 ± 608	278 ± 233	1026 ± 680	1976 ± 1298	1467 ± 1071	5549 ± 2149
Ephemeroptera	GC	12 ± 10	83 ± 98	17 ± 16	39 ± 4	110 ± 52	68 ± 59	266 ± 231	197 ± 228	210 ± 115	654 ± 270	1155 ± 944	1716 ± 1127
Heteroptera	P	6 ± 11	0 ± 0	0 ± 0	6 ± 11	28 ± 24	20 ± 35	11 ± 19	12 ± 21	0 ± 0	0 ± 0	46 ± 42	0 ± 0
Odonata	P	19 ± 33	0 ± 0	0 ± 0	0 ± 0	0 ± 0	16 ± 27	0 ± 0	0 ± 0	0 ± 0	14 ± 24	19 ± 33	0 ± 0
Trichoptera	Sh/FC/GC/Sc/P	6 ± 11	0 ± 0	0 ± 0	25 ± 9	113 ± 143	147 ± 51	104 ± 84	63 ± 52	106 ± 128	188 ± 149	80 ± 33	421 ± 364
Nematoda	P	0 ± 0	0 ± 0	0 ± 0	0 ± 0	7 ± 12	16 ± 27	0 ± 0	0 ± 0	0 ± 0	14 ± 24	19 ± 33	0 ± 0
Total Density		827	586	101	1401	1064	1555	1798	550	1616	3397	3556	9676
Diversity H′		0.136	0.201	0.197	0.191	0.633	0.568	0.570	0.454	0.494	0.571	0.624	0.555

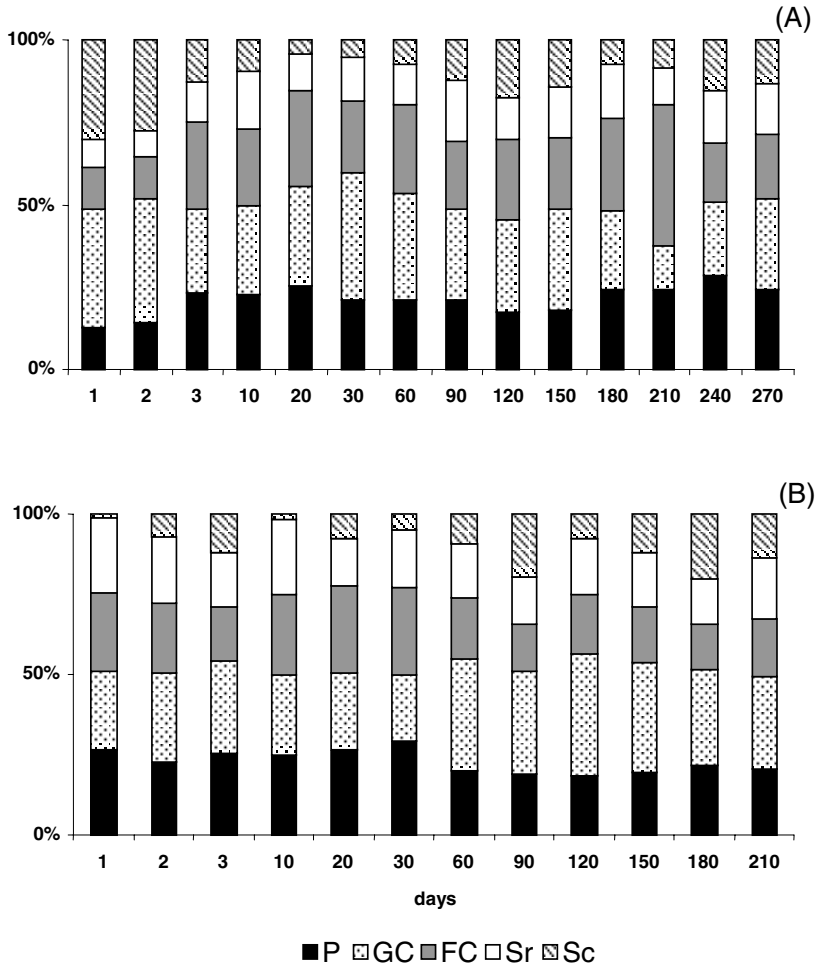

Figure 2. Abundance of functional feeding groups during detritus breakdown in the 3rd (A) and 4th (B) order reach. P = Predators, FC = Filtering-collectors, GC = Gathering-collectors, S = Shredders, Sc = Scrapers.

observed in the final stages of breakdown on the 210th day in the 4th order and in the 270th day in the 3rd order reach (Fig. 4C). However, differences found in the densities of bacteria, fungi, and, yeasts proved not to be significant between the 3rd and 4th order reaches (ANOVA, $F = 0.92$ $p > 0.05$).

4. Discussion

4.1. Decomposition Process

Based on PETERSEN and CUMMINS (1974) the decomposition coefficient can be classified (rapid, $k > 0.01$ d^{-1}; intermediate, 0.01 $d^{-1} > k > 0.005$ d^{-1} and slow $k < 0.005$ d^{-1}) in the 4th

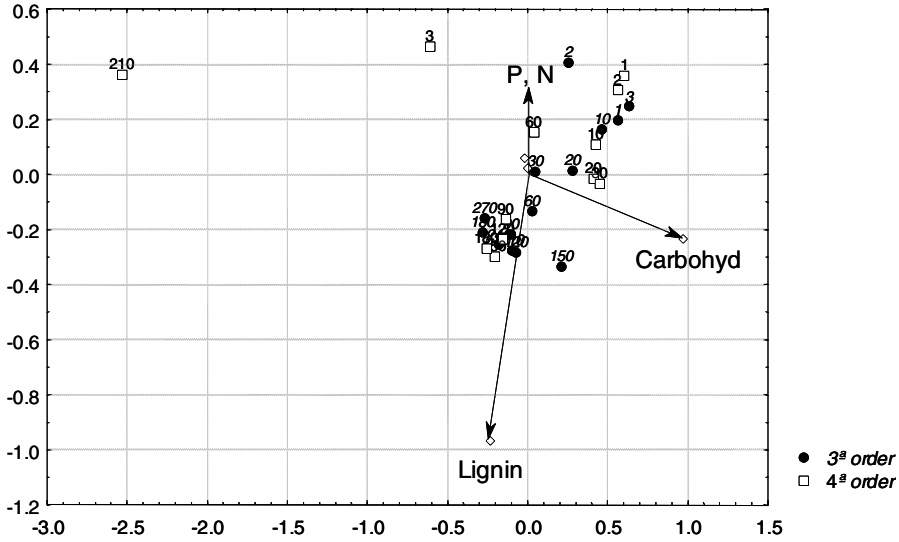

Figure 3. Ordination of invertebrates colonization in the 3rd (circle) and 4th (square) order reaches during detritus breakdown (days = numbers) according to the chemical composition variables (vectors) using canonical correspondence analysis (CCA).

order reach as rapid and in the 3rd order reach as intermediate. For both reaches of the stream the breakdown rates were very similar to those found for other species in temperate regions (ABELHO, 2001) and tropical regions (MATHURIAU and CHAUVET, 2002; MOULTON and MAGALHÃES, 2003). However, the decay rates found in this study were less than those found for leaves of native African species (DOBSON *et al.*, 2003).

While the initial chemical composition of the detritus is more important for breakdown (HOORENS *et al.*, 2003), some molecules (e.g., proteins and carbohydrates) can facilitate, while others (e.g., lignin and phenols) can constrain this process (SUBERKROPP *et al.*, 1976; OSTROFSKY, 1997; OSONO and TAKEDA, 1999 and 2001). In our study the chemical composition of detritus was not important in evaluating leaf breakdown, thus we incubated detritus in the two reaches studied with the same chemical characteristics.

Contrary to that which was observed in the chemical composition of the detritus, some abiotic parameters of the waters tested differed in the two reaches of the stream. The stream water velocity, in the 4th order reach, was 1.5 times higher than in the 3rd order, this difference increased with the onset of rains. Stream water velocity influences decomposition through the physical abrasion and oxygenation of water (we found significant differences) which facilitates breakdown of the detritus (WEBSTER and BENFIELD, 1986). The concentration of nitrate in the water was also higher in the 4th order reach than in the 3rd order reach. The availability of nutrients in the water has a strong influence on microbial colonization, possibly also affecting decomposition (SUBERKROPP and CHAUVET, 1995; ROSEMOND *et al.*, 2002; CHADWICK and HURYN, 2003; GULIS and SUBERKROPP, 2003). Therefore, the environmental differences (physical and chemical) observed here indicate the strong influences that these factors had over the decay rates found in both reaches, as was observed in CASAS and GESSNER (1999) in the Mediterranean.

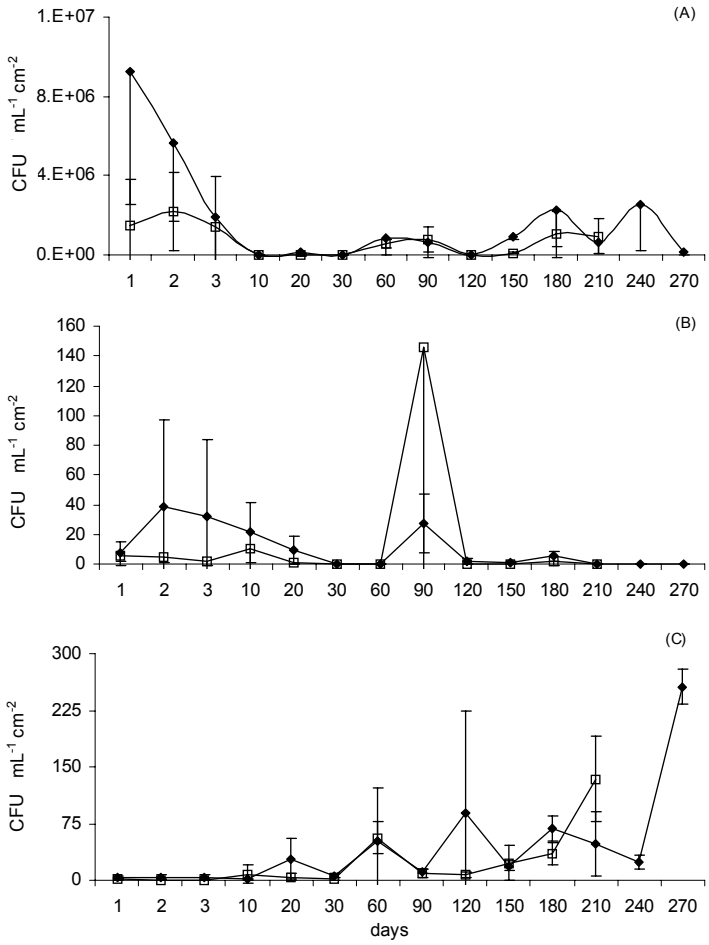

Figure 4. Densities (means of three replicates ± standard deviation) of Heterotrophic Bacteria (A), Yeast (B) and Filamentous Fungi (C) during detritus breakdown in the 3rd (diamond) and 4th (square) order reaches of Indaiá stream.

4.2. Biological Colonization

Biological influences are also important factors that may affect the rate of decomposition (GESSNER *et al*., 1999), through the actions of invertebrates (mainly shredders) and microorganisms (fungi). Chironomidae and Ephemeroptera were the main groups of invertebrates present during the entire decomposition process, confirming recent observations carried out in streams (MATHURIAU and CHAUVET, 2002; MOULTON and MAGALHÃES, 2003) and lakes (NESSIMIAN and DE LIMA, 1997) situated in tropical regions. These two groups could be responsible for structuring the entire invertebrate community due to their ability to colonize, independently of quality and/or time of decomposition, different leaf detritus. These results corroborate those obtained in previous studies which used aquatic macrophyte detritus from a coastal lake (GONÇALVES *et al*., 2000 and 2003). Furthermore, the invertebrate communi-

ty appears not to have been structured by the decomposition process, which would indicate that this community was organized and structured by the degradative ecological succession process (GONÇALVES *et al.*, 2004).

In our study we observed that the chemical composition of the detritus influenced invertebrate colonization. This may be explained by the time it takes each detritus to become more palatable to invertebrates. Furthermore, it could be an indication of the importance of microorganisms in increasing the palatability of leaf detritus for invertebrates (ROSEMOND *et al*., 1998; GRAÇA *et al*, 2001). Some evidence in our study indicates this effect: the detritus in the 3rd order reach showed a higher diversity and density of invertebrates, where the density of heterotrophic bacteria, yeasts, and filamentous fungi tended to also be higher. Shredders tended to be more abundant in the final stages of decomposition, during which an increase in microorganisms was also observed.

The microbial community is of fundamental importance, throughout the world, in releasing the energy stored in detritus (GULIS and SUBERKROPP, 2003) mainly in tropical regions where shredders are rare (Irons *et al*., 1994; Dobson *et al*., 2002). According to SRIDHAR and BÄRLOCHER (2000) and SUBERKROPP (2001) the biomass of fungi associated with detritus was higher than that of bacteria during leaf breakdown, indicating that fungi were the main primary decomposers, accounting for up to 17% of the total biomass of all detritus. In the present study, the highest counts of bacteria in the initial stages of leaf breakdown suggest that bacteria are more important during this period. These results contrast with other results in temperate streams. SAMPAIO *et al.* (2001) found the highest counts of bacteria in advanced stages of breakdown. Other authors showed that bacteria colonization (measured by biomass) have relatively no importance when compared with fungi in the initial detritus breakdown (WEYERS and SUBERKROPP, 1996; JUGNIA *et al*., 2000; GRAÇA, 2001). Our results suggest that bacteria metabolize the protein and simple sugars of the detritus during the initial stages and that fungi act on the degradation of complex polysaccharides, mainly those present in the advanced stages. We noted that generally, when counts of bacteria decrease, counts of filamentous fungi increase and this can be explained, as suggested by SUBERKROPP and KLUG (1976) and CHAMIER *et al.* (1984), by the fact that fungi and bacteria compete with each other for nutrients.

Concerning yeast colonization, it was not possible to identify their effective role in leaf breakdown. As described by SAMPAIO *et al*. (2001), there are several references which show the capacity of yeasts in the degradation of the principal constituents of plants, for example: cellulose degradation and xilanase activities. Our results showed fluctuation in the density of yeasts and according to SAMPAIO *et al*. (2001), who found similar results for yeast counts, this group of fungi presumably are opportunistic organisms assimilating substances released during degradation by other fungi or bacteria.

In summary, we found significant difference in breakdown rates between the reaches studied but not in their biological communities. As colonization of invertebrates and microorganisms seem to be different in tropical and temperate streams, and most of the information available is from the temperate zone, more experiments are needed to elucidate the differences in leaf breakdown in these regions.

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