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Effects of nitrate enrichment on leaf litter decomposition

Efeitos do enriquecimento por nitrato sobre a decomposição de detritos foliares

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Abstract: Aim: This study aimed to determine the effects of nitrate enrichment on leaf decomposition process and the kinetic parameters of decomposition model; **Methods:** Samples of water from a first-order stream and senescent leaves of the native tree species *Campomanesia xanthocarpa* O. Berg (Myrtaceae) were collected in South of Brazil. The leaves were oven-dried, grounded and for each experimental condition (control and enriched) 20 decomposition chambers were prepared with leaf fragments and unfiltered stream water (with and without nitrate addition), maintained under low and high oxygen conditions. In sampling days the particulate and dissolved organic carbon and total inorganic carbon concentrations were evaluated; **Results:** The decomposition of particulate and dissolved organic carbon (POC and DOC) was faster in nitrate enriched treatment under high dissolved oxygen condition. The DOC mineralization coefficients (k_3) were in average 283-fold higher than the rate constants for refractory POC (RPOC) mineralization, being the enriched k_3 2.3-fold higher than the control k_3; **Conclusions:** The leaf litter decomposition was affected by dissolved nitrate concentration in the water and RPOC and DOC decomposition was faster with nitrate enrichment than in reference natural conditions (without nitrate enrichment). Thus, dissolved nitrate seems to be an important factor in controlling litter decomposition and its increase affects the leaf carbon processing in stream ecosystems.

Keywords: nutrients, kinetic model, organic carbon, eutrophication.

Resumo: Objetivo: Esse estudo tem por objetivo determinar os efeitos do enriquecimento por nitrato sobre o processo de decomposição foliar e os parâmetros cinéticos do modelo de decomposição; **Métodos:** Foram coletadas amostras de água de um riacho de primeira-ordem e folhas senescentes da espécie arbórea nativa *Campomanesia xanthocarpa* O. Berg (Myrtaceae) no sul do Brasil. As folhas foram secas em estufa, moídas e para cada condição experimental (controle e enriquecido), 20 câmaras de decomposição foram preparadas com fragmentos foliares e água não filtrada do riacho (com e sem adição de nitrato), mantidas sobre condições de baixa e alta oxigenação. Nos dias amostrais, as concentrações de carbono orgânico particulado e dissolvido e, carbono inorgânico total foram avaliadas; **Resultados:** A decomposição do carbono orgânico particulado e dissolvido (COP e COD) foi mais acelerada no tratamento enriquecido com nitrato e sob a condição de alta oxigenação. O coeficiente de mineralização do COD (k_3) foi em média 283 vezes maior que as taxas de mineralização das frações de carbono orgânico particulado refratário (COPR), sendo o k_3 do meio enriquecido 2,3 vezes maior que o k_3 do controle; **Conclusões:** A decomposição dos detritos foliares foi afetada pela concentração de nitrato dissolvido na água e as decomposições do COPR e COD foram mais aceleradas com o enriquecimento por nitrato que em condições naturais de referência (sem enriquecimento por nitrato). Assim, o nitrato dissolvido mostrou ser um importante fator no controle da decomposição de detritos e seu aumento afeta a ciclagem de carbono foliar em ecossistemas de riachos.

Palavras-chave: nutrientes, modelo cinético, carbono orgânico, eutrofização.

1. Introduction

Low order streams are dependent on inputs of organic material produced by trees in the riparian zone, particularly in the form of leaf litter (Wallace et al., 1997). The leaf breakdown in streams is controlled by leaf characteristics (the structural and chemical compounds) and environmental factors, such as temperature (Mellilo et al., 1984), the activity of invertebrates, the concentration of dissolved oxygen and nutrients in the stream water (Webster and Benfield, 1986; Boulton and Boon, 1991). The dissolved nutrients are important to leaf decomposition due to regulating the leaf-decaying fungi activity (Suberkropp and Chauvet, 1995). Thus, high nutrients concentration can stimulate the activity of heterotrophic microorganism on litter and, consequently, the decomposition rates of leaf litter (Gessner and Chauvet, 1994).

Eutrophication caused by anthropogenic disturbance can affect the organisms and ecosystem functioning through increased nutrient concentrations and indirectly through oxygen depletion. However, the oxygen depletion can mainly occur in slow flowing streams due to bacterial carbon oxidation (Nijboer and Verdonschoc, 2004). The major concern in streams is sources of inorganic nitrogen from urban activities and agriculture fertilizers (Carpenter et al., 1998). Much of the nitrogen from these sources goes on to enter streams, primarily as nitrates, and is transported downstream. Several studies have indicated that leaf decomposition and associated microorganism in stream ecosystems can be affected by the concentration of nutrients (Suberkropp and Chauvet, 1995; Grattan and Suberkropp, 2001; Gulis and Suberkropp, 2003a). The nutrient enrichment effects on litter decomposition have focused on i) field studies, with litter bags or streams experimentally enriched (Robinson and Gessner, 2000; Gulis and Suberkropp, 2003a; Gulis et al., 2004; Ferreira et al., 2006); and ii) laboratory studies, with aquatic macrophytes from reservoirs (Lemos and Bianchini Junior, 1998; Lemos et al., 2007) or leaves from riparian vegetation of streams (Howarth and Fisher, 1976; Fairchild et al., 1984). Although a number of field studies have examined the effects of nitrogen enrichment on decomposition rates (Meyer and Johnson, 1983; Newbold et al. 1983; Abelho and Graça, 2006; Ferreira et al., 2006) many of them have produced varying results. In addition, field experiments may vary not only in nitrogen content, but also in other environmental factors.

This study aimed to determine the effects of nitrate enrichment on leaf decomposition process and the kinetic parameters of the decomposition model. We tested the effects of dissolved nitrate, separately from other environmental factors, on *Campomanesia xanthocarpa* O.Berg litter decomposition under lab-controlled conditions of temperature and oxygen. We predicted that nitrate enrichment would stimulate the decomposition of particulate and dissolved organic carbon. The decomposition model attempts to analyze the leaf decomposition in an ecosystem perspective in relation to the nitrates increases caused by anthropogenic disturbances. This model also provides information to leaf litter decomposition understanding and can be used to water quality evaluation.

2. Material and Methods

2.1. Water and leaf litter sampling

Samples of water were collected in a first-order stream of Suzana River basin in South of Brazil (27° 36' 43.5" S and 52° 14' 05.4 W; 734 m a.s.l.), with subtropical regional climate (Budke et al., 2010). The stream is classified as oligotrophic system with relatively low anthropogenic interferences, low concentrations of dissolved organic carbon (8.6 ± 2.2 $mg.L^{-1}$) and nitrate (1.12 ± 0.53 $mg.L^{-1}$), and high concentration of dissolved oxygen (>7 $mg.L^{-1}$) comparing with reference sites in the region (Hepp and Santos, 2009; Milesi et al., 2009). The stream presents circumneutral pH (6.8 ± 0.2), electrical conductivity of 30.6 ± 5.3 $\mu S.cm^{-1}$ and water temperature of 20 ± 1 °C (November/2011). It was ~0.3 m wide and ~0.1 m deep. The riparian vegetation included *Sebastiania brasiliensis* Spreng., *Cupania vernalis* Cambess., *Casearia sylvestris* Sw., *Campomanesia xanthocarpa* O. Berg. and other native common trees (Trevisan and Hepp, 2007; Hepp et al., 2008).

Senescent leaves of the native tree species *Campomanesia xanthocarpa* O. Berg (Myrtaceae) were collected from one forest fragment, in the North of Rio Grande do Sul State (27° 36' S and 52° 13' W), in spring 2010. The leaves were oven-dried (35 °C/24-48 hours), grounded (1 mm mesh), homogenized and stored until incubation. The species choice was based in their expressive dispersion in the region and their occurrence in the riparian vegetation of subtropical streams (Oliveira-Filho et al., 2006). The *C. xanthocarpa* leaves presented in their chemical composition 93.26 ± 0.61% DM of organic matter, 2.08 ± 0.10% DM of nitrogen, 0.04 ± 0.01% DM of tannins and the C:N rate of 22.58 ± 0.92% DM (Tonin et al., submitted), being characterized as low-quality litter. The stimulation effect of nutrient enrichment is more pronounced for low-quality detritus (i.e., high C:N rates, high concentrations of secondary compounds as tannins and structural compounds) (Gulis et al., 2004).

2.2. Decomposition experiments

Decomposition chambers (n = 80) were prepared in laboratory divided in i) control treatment, with unfiltered stream water without nitrate addition (nitrate concentration average: 1.12 ± 0.53 $mg.L^{-1}$) and leaf fragments; and, ii) enriched treatment, with unfiltered water with nitrate addition (1.33 $g.L^{-1}$ $NaNO_3$) (nitrate concentration average: 12.17 ± 0.20 $mg.L^{-1}$) and leaf fragments. In each chamber 0.50 ± 0.01 g (on dry mass basis) of leaf fragments were added to 100 mL of unfiltered stream water. The chambers were maintained under high dissolved oxygen (n = 20 control treatment, n = 20 enriched treatment) and low dissolved oxygen (n = 20 control treatment, n = 20 enriched treatment) conditions. The incubations were performed in the dark at 20 ± 1 °C (representing the annual water average temperature). High dissolved oxygen conditions (7.9 ± 0.1 $mg.L^{-1}$) were maintained by constant bubbling air flux while low dissolved conditions (4.1 ± 0.1 $mg.L^{-1}$) were obtained by absence of bubbling air flux. These experiment conditions of temperature and dissolved oxygen were based on physical and chemical characteristics of Alto Uruguai region streams (König et al., 2008; Hepp and Santos, 2009; Hepp et al., 2010, Hepp and Restello, 2010).

Eight chambers of each treatment were retrieved from the incubations on days 1, 7, 15 and 22 to decomposition process analyses. Were evaluated the temporal variations of particulate organic carbon (POC), dissolved organic carbon (DOC) and total inorganic carbon (TIC). The ash-free dry mass (AFDM) was obtained through organic matter ignition (550 °C/4 hours). The POC and DOC were estimated from POC: total detritus = 0.47 × AFDM and total organic carbon analyzer, TOC-VCSH (Shimadzu®), respectively. To DOC determinations, the samples were pre-filtered (Millipore 0.45 µm). The method to determine the carbon fractions consists in a 20 µL combustion sample at 680 °C to CO_2 conversion. The total inorganic carbon (TIC) derived from organic matter mineralization was calculated by the difference between the initial POC and the remaining organic carbon determined in the sampling days (POC + DOC).

2.3. Leaf decomposition kinetics and data analysis

In order to describe the mineralization of *C. xanthocarpa* litter, a set of equations was used (Equations 1 to 3) (Bianchini Junior, 2003). The parameterizations were obtained by fitting the temporal variations of POC and DOC. These fittings were performed using nonlinear regressions with the iterative algorithm of Levenberg-Marquardt (Press et al., 1993).

The POC mass loss (leaching and mineralization process of labile and refractory compounds related with particulate carbon) was determined from (Equation 1):

$$dPOC/dt = -k_T LSPOC - k_4 POC_R \qquad (1)$$

where: POC = particulate organic carbon; LSPOC = labile and soluble fractions of particulate organic carbon; RPOC = refractory fractions of particulate organic carbon; k_T = global mass loss coefficient from LSPOC (day^{-1}); $k_T = k_1 + k_2$ (k_1 = mineralization coefficient from LPOC (day^{-1}); k_2 = leaching coefficient from LSPOC (day^{-1})); k_4 = RPOC mineralization coefficient (day^{-1}). Formation and mineralization of DOC (Equation 2):

$$dDOC/dt = k_T LSPOC - k_3 DOC \qquad (2)$$

where: DOC = dissolved organic carbon; k_3 = DOC mineralization coefficient (formation of inorganic substances and CO_2 (day^{-1})). Formation of inorganic substances (mineralization) (Equation 3):

$$dTIC/dt = k_T LPOC + k_4 RPOC + k_3 DOC \qquad (3)$$

where: TIC = total inorganic carbon.

The half time ($t_{1/2}$) corresponding to the rates of the various process (leaching, LSPOC, DOC and RPOC oxidations) were estimated through Equation 4.

$$t_{1/2} = \ln(0.5)/-k \qquad (4)$$

where: k = constant coefficients for the process (leaching or mineralization).

To assess the differences in POC, DOC and TIC (log [x+1]) between treatments, oxygen conditions and sampling days were used covariance analyses (ANCOVA). The analyses were performed using the MASS packaged (Venables and Ripley, 2002) from R software (R Development Core Team, 2010).

3. Results

During the laboratory assays, we found that decomposition of POC and DOC was faster in nitrate enriched treatment under high dissolved oxygen condition. Overall, the POC fraction presented significant variations ($F_{(3, 48)} = 25.48$, $P < 0.001$) between the two treatments ($P < 0.001$) and sampling days ($P < 0.001$), but not between oxygen conditions ($P = 0.152$). The DOC concentration was not statistically different ($F_{(3, 48)} = 2.626$, $P = 0.060$) between treatments ($P = 0.333$) and oxygen conditions ($P = 0.333$), while it was significantly different among sampling days ($P = 0.021$), as a consequence of LSPOC mass loss, reaching maximum values (1.27-2.63%) in the first day of experiment. After the predominance of the leaching (DOC; Figure 1), in all the incubations, the DOC concentration tended to decrease. The mineralization of DOC was statistically faster in the nitrate-enriched treatments ($F_{(3, 48)} = 91.12$, $P < 0.001$), differing among sampling days ($P < 0.001$) and oxygen conditions ($P < 0.001$).

The values of LSPOC, RPOC, k_T, k_3 and k_4 calculated for the proposed model (Equations 1 to 3) are show in Table 1. The kinetics model fitted the experimental data with high determination coefficients ($r^2 = 0.90–0.99$). The evolution of POC, DOC and TIC indicates that DOC mineralization (k_3) was in average 283-fold higher than the rate constants for RPOC mineralization. Comparing DOC mineralization coefficients, it can be observed that enriched k_3 was in average 2.3-fold higher than the control k_3. The $k_3–t_{1/2}$ varied for the control treatment from 25 (low oxygen) to 21 days (high oxygen) and from 8 (low oxygen) to 13 days (high oxygen) for the enriched treatment. In contrast, RPOC mineralization coefficients were in average

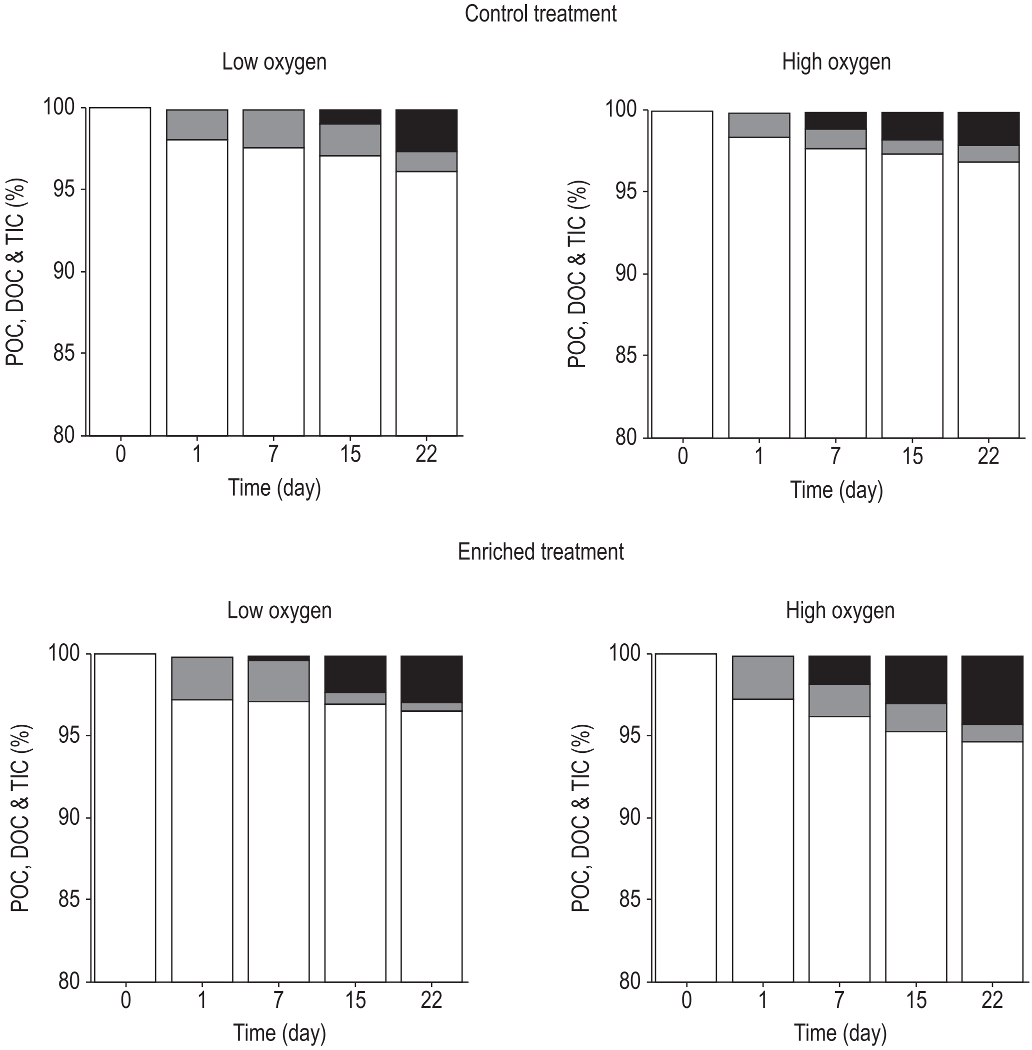

Figure 1. Temporal variations of POC (white bars), DOC (grey bars) and TIC (black bars) from *C. xanthocarpa* leaf litter decomposition in enriched and control treatment under low and high oxygen conditions.

Table 1. Parameters of kinetic model from *Campomanesia xanthocarpa* leaf litter decomposition in control and enriched treatment under low and high oxygen conditions.

	Control treatment				Enriched treatment			
	Low oxygen		High oxygen		Low oxygen		High oxygen	
		Error		Error		Error		Error
LSPOC (%)	2.1	0.49	2.0	0.13	3.0	0.56	3.2	0.24
k_T (day^{-1})	1.5	–	1.5	–	1.5	–	1.5	–
k_T – $t_{1/2}$ (day^{-1})	0.46	–	0.46	–	0.46	–	0.46	–
RPOC (%)	97.9	0.33	98.0	0.09	96.9	0.38	96.8	0.16
k_4 (day^{-1})	0.0007	0.0002	0.0005	0.0001	0.0001	0.0003	0.0010	0.0001
k_4– $t_{1/2}$ (day^{-1})	962.7	–	1359.1	–	7701.6	–	693.1	–
r^2	0.97	–	0.99	–	0.96	–	0.99	–
DOC (%)	1.5	0.91	1.3	1.19	0.9	0.57	1.5	0.99
k_3 (day^{-1})	0.0279	0.0067	0.0337	0.0124	0.0880	0.0153	0.0517	0.0113
k_3– $t_{1/2}$ (day^{-1})	24.9	–	20.6	20.6	7.9	–	13.4	–
LPOC (%)	0.6	–	0.7	–	2.1	–	1.7	–
r^2	0.97	–	0.90	–	0.96	–	0.94	–

LSPOC (labile and soluble fractions of particulate organic carbon), RPOC (refractory fractions of particulate organic carbon), k_T (global mass loss coefficient from LSPOC) k_T (k_1 + k_2 [k_1 = mineralization coefficient from LPOC, k_2 = leaching coefficient from LSPOC]), k_3 (DOC mineralization coefficient), k_4 (RPOC mineralization coefficient).

1.1-fold higher in the control treatment than in the enriched treatment.

In the *C. xanthocarpa* leaf tissues, the refractory particulate organic carbon (RPOC) fraction showed the predominance over the POC labile/soluble fractions (LSPOC). The amount of RPOC was 97.4% while LSPOC represented only 2.6%. From the fitting of POC kinetics, the global decay coefficients (leaching mineralization; LSPOC fractions) were higher (k_T = 1.5 day^{-1}), corresponding to a half-time ($t_{1/2}$) of ~11 hours, regardless of treatment and oxygen condition. The LSPOC mass loss was shown to be faster than other reactions such as DOC and RPOC mineralization.

4. Discussion

This study showed that nitrate enrichment directly stimulated leaf litter decomposition in lab-controlled conditions. Such stimulation of leaf litter decomposition by increased nitrogen in water has been shown previously in laboratory microcosms (Howarth and Fisher, 1976; Fairchild et al., 1984). In field studies, however, nitrate enrichment has had variable effects on leaf decomposition rates. Triska and Sedell (1976) and Grattan and Suberkropp (2001) found no significant effects of nitrate addition on leaf litter processing while stimulation effects were demonstrated by Nikolcheva and Bärlocher (2005) and Ferreira et al. (2006). However, the decomposition rates of the present study were comparable mainly to the obtained in laboratory experiments due to exclusion of environmental factors involved in litter decomposition in streams (mainly activity of shredder invertebrates and current mechanical abrasion). Considering the leaf litter as heterogeneous substrate, it is possible to admit that the litter is constituted by two fractions of particulate organic carbon: i) labile and/or soluble (LSPOC) and ii) refractory (RPOC). Based on these fractions (LSPOC and RPOC), generally, the POC and DOC decomposition was faster in nitrate enriched treatments, regardless of oxygen condition. Nevertheless, nitrate enrichment had a pronounced influence on DOC than POC decomposition. However, Lemos and Bianchini Junior (1998) and Lemos et al. (2007) in laboratory experiments with aquatic macrophytes observed that POC decomposition was stimulated by nitrate enrichment instead of DOC.

In the first days of decomposition, the dissolution of soluble compounds and oxidation of labile compounds prevailed, being responsible for the initial mass loss and chemical changes of leaf litter. These fast processes of leaf mass loss are associated with release of cytoplasm fractions and the hydrosoluble structural compounds (Canhoto and Graça, 1996). The leaching period generally varies from the first 24 hours to 15 days (Brum and Esteves, 2001) depending on variables such as water temperature, water flow, chemical composition of leaf species and experimental procedures (oven dry of leaf litter) (Abelho, 2001). The LSPOC and RPOC proportions indicate that the *C. xanthocarpa* litter contained mainly refractory fractions formed by compounds that are strongly resistant to enzymatic degradation (e.g. lignin, cellulose and hemicellulose) (Gessner and Chauvet, 1994). The predominance of refractory fractions in the leaf litter suggests that structural compounds can play a direct role in determining leaf decomposition by inhibiting microbial consumers. The presence of recalcitrant structural compounds, which require specialized enzymes to be degraded, can limit microbial respiration on leaves (Ardón et al., 2006). These characteristics of refractory fractions are responsible for the slow decomposition of RPOC in control and enriched treatment (k_4 range: 0.0001-0.0010) comparing with the fast mineralization of DOC, in both treatments (k_3 range: 0.0279-0.0880). In this context, according to 83 experiments (accomplished in streams with leaves of tree species) the decomposition rates (k) vary from 0.0002 day^{-1} ($t_{1/2}$ = 3465 days) to 0.1980 day^{-1} ($t_{1/2}$ = 4 days) (Gimenes et al., 2010). Overall, according to this revision, the rates of RPOC decomposition obtained in this study were lower than most of the observed to leaf litter decomposition in field experiments. However, the coefficients of DOC loss ($k_3.day^{-1}$) can be considered fast compared to those obtained by Lemos and Bianchini Junior (1998) and Lemos et al. (2007) in laboratory experiments with nitrate enrichment on aquatic macrophytes decay (k_3: 0.002-0.005 day^{-1}, k_3: 0.012-0.025 day^{-1}, respectively). According to Petersen and Cummins (1974), the coefficients of RPOC and DOC decomposition can be classified as slow ($k < 0.005$ day^{-1}) and fast ($k > 0.010$ day^{-1}), respectively. Nevertheless, due to experimental differences (chemical composition of leaf litter, nitrate concentration, temperature and oxygen conditions) between laboratory and field studies, the decomposition coefficients (k_T, k_3 e k_4) must be compared carefully.

In relation to oxygen availability, we may suggest that the microorganism involved in refractory fractions decomposition showed similar degradation efficiency between oxygen conditions in control treatment while the k_4 values for the enriched treatment suggest that microbial degradation of structural compounds was faster on high oxygen conditions. Among the microorganism involved, the fungi (mainly aquatic hyphomycetes) play a major role on microbial decomposition of leaf litter through the production of enzymes that are capable of degrading structural compounds of leaves, whereas bacteria increase their importance only after the partial degradation of leaf litter material (Baldy et al., 1995; Weyers and Suberkropp, 1996). The positive response of fungi to nutrient enrichment (mainly nitrogen) was demonstrated in streams (Gulis and Suberkropp, 2003a) and in microcosms experiments (Gulis and Suberkropp, 2003b, 2003c). However, the fungi activity on leaves is affected also by other environmental factors as dissolved oxygen concentration. Medeiros et al. (2009) observed that decomposer fungi and litter decomposition were strongly affected by a decrease in dissolved oxygen in the water (to 7.6 $mg.L^{-1}$). These results clarify the importance of oxygen content in the water to leaf litter processing and suggest that this process, in the control treatment, was limited by dissolved nitrogen concentration.

In conclusion, the leaf litter decomposition was affected by dissolved nitrate concentration in the water. As expected, the RPOC and DOC decomposition was faster with nitrate enrichment than in reference natural conditions (without nitrate enrichment). Therefore, dissolved nitrate seems to be an important factor in controlling litter decomposition, mainly associated with high oxygen concentrations in the water, and its increase affects the leaf carbon processing. Thus, we may suggest that one of the major effects of anthropogenic eutrophication on streams would be to increase the decomposition rate of leaf litter. This should reduce the amount of organic matter in the stream and interfere profoundly on ecosystem functioning.

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