Internat. Rev. Hydrobiol.	**96**	2011	3	209–220

DOI: 10.1002/iroh.201111332

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Research Paper

Phosphorus Enrichment Affects Immobilization but not Litter Decomposition or Exoenzymatic Activities in a Pampean Stream

key words: Typha, decay, leaf decomposition, invertebrate abundance

Abstract

The effect of phosphorous enrichment on decomposition rate, exoenzymatic activities (β-glucosidase, cellobiohydrolase and alkaline phosphatase), and macroinvertebrate abundance in *Typha latifolia* leaves were assessed in a 2nd order Pampean stream (Central Argentina). Phosphorous was added to a downstream reach while another reach located upstream was kept intact and, once significant differences in phosphorus concentration in water were attained, leaf bags were attached to each reach bottom. *T. latifolia* leaves lost 77% of their initial weight along 154 days and decomposition rates were not significantly different between reaches. Besides, neither exoenzymatic activities nor macroinvertebrate abundances differed between reaches. However, an increment in leaf phosphorus content, attributed to immobilization by decomposer microorganisms, was detected in the enriched reach.

1. Introduction

In aquatic ecosystems, dissolved nutrients, particularly phosphorus (P) and nitrogen (N), are important regulators of biotic processes such as primary production (Grimm and Fisher, 1986), growth (Hart and Robinson, 1990; Rosemond *et al.*, 1993), and decomposition (Peterson *et al.*, 1993). P and N concentrations are routinely affected by human activities and are expected to increase in the future, as global warming will increase runoff and sediment load to fluvial ecosystems of Africa and South America (Vitousek *et al.*, 1997; Chang *et al.*, 2001; Rodrigues Capítulo *et al.*, 2010). Thus the quantification of the impact of high variations in nutrient concentrations on aquatic ecosystems is a task of interest for basic and applied science; particularly in low order lotic ecosystems with great value as biodiversity reservoirs located in areas with an intense human activity, like the Pampean plain (Giorgi *et al.*, 2005).

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Over the last fifty years, the attention directed to decomposition process has increased as forest streams, which are heterotrophic, take a major part of its energy from allochthonous sources (Gregory *et al.*, 1991; Wallace *et al.*, 1999). Pampean streams generally lack a riparian forest, which in addition to the low current velocities and high nutrient levels in Pampean streams allow the development of dense and diverse macrophyte communities (Feijoó and Lombardo, 2007) that decay in winter, supplying important amounts of coarse particulate organic matter to the system. Since this aquatic vegetation is practically not consumed by animals, decomposition would be the only process which allows the return of this material to the system. Hence, although the input of allochthonous material is negligible in Pampean streams, decomposition may still play a key role on the matter recirculation.

There is a general consensus on the existence of a positive relationship between decomposition rate and P and N substrate concentrations (Carpenter and Adams, 1979; Enriquez *et al*., 1993; Chadwick and Huryn, 2003), and a similar effect has been reported for the increase of dissolved nutrients in field (Elwood *et al*., 1981; Peterson *et al.*, 1993; Suberkropp and Chauvet, 1995; Robinson and Gessner, 2000; Grattan and Suberkropp, 2001; Gulis and Suberkropp, 2003a) and laboratory experiments (Carpenter and Adams, 1979; Sridhar and Bärlocher, 2000; Gulis and Suberkropp, 2003b). However, in some studies these positive effects have been caused only by N but not by P concentrations, either in the substrate (Carpenter and Adams, 1979) or in water (Carpenter and Adams, 1979; Suberkropp and Chauvet, 1995). Finally, stream geomorphology (Meyer, 1980), water temperature (Carvalho *et al.*, 2005), dissolved oxygen (Cummins *et al.*, 1980), substrate (Alonso *et al.*, 2010), and shredder density (Sponseller and Benfield, 2001) may also influence the rate of leaf breakdown in streams.

Decomposition process of leaves in streams consists of three general phases: i) leaching of soluble compounds, ii) microbial colonization and degradation (conditioning); iii) fragmentation by physical abrasion and invertebrate shredding (Webster and Benfield, 1986).

With respect to heterotrophic microorganisms, these tend to have a very high phosphorus and nitrogen content indicative of high requirements for these nutrients (Goldman *et al.*, 1987; Vadstein and Olsen, 1989). According to this, bacterial growth efficiency has been found to increase with the increment of C/P and N/P ratios in substrate (Goldman *et al.*, 1987). In addition, high fungal biomass concentrations and activities have been found in streams with high dissolved nutrient concentrations (Suberkropp and Chauvet, 1995; Methvin and Suberkropp, 2003). Finally, an increase in water nutrient concentrations has positively affected fungal biomass and bacterial activity, in field (Gulis *et al.*, 2004) and laboratory (Gulis and Suberkropp, 2003b) experiments. Positive effects, of N but not of P, have been found for fungal sporulation rate (Abelho and Graça, 2006). Then, detritus with high nutrient content or located in environments with high dissolved nutrient concentrations are expected to decompose fast, due to a high growth of the microbial decomposer communities.

Decomposing organisms (bacteria and fungi) are known to produce a wide range of exoenzymes capable of mineralizing organic matter (Arnosti, 2003), and the activity of several of these enzymes correlates consistently with the decomposition rate of organic matter in continental aquatic ecosystems (Sinsabaugh, 1994; Sinsabaugh *et al.*, 1994). For this reason, exoenzymatic activities are used as a reliable proxy of microbial decomposition (Jackson *et al.*, 1995; Kim and Rejmankova, 2004). In fact, decomposition can be thought as an emergent process of exoenzymatic activities that are regulated by site-specific factors such as temperature, moisture and nutrient availability, and secondarily by litter chemistry through adsorption, inhibition and stabilization processes (Sinsabaugh, 1994).

Regarding invertebrates, detritivores classified as shredders are known to feed primarily on big pieces of vegetal detritus and their associated microorganisms (Cummins, 1973). In fact, studies suggest that some of them may be assimilating mainly the microbial biomass

(CUMMINS and KLUG, 1979; CHUNG and SUBERKROPP, 2009). Then, the effect of dissolved nutrient on the decomposition rate may be mediated primarily by heterotrophic microorganism (through conditioning) and secondarily by shredder invertebrates (through fragmentation). However, positive effects of enrichment over shredder abundance independent from microbial biomass have also been reported (ROBINSON and GESSNER, 2000).

The main objective of this work was to test the hypothesis that an increment in dissolved phosphorus in water will increase the decomposition rate of autochthonous material in a Pampean stream. In addition, the effects of artificial phosphorus enrichment on exoenzymatic activities and macroinvertebrate abundances associated with decomposing vegetal material were also analyzed.

2. Materials and Methods

2.1. Study Site

The study was conducted between September 2008 and March 2009, at two reaches of the La Choza stream (34°44′ S, 59°06′ W), a second order stream tributary of the Luján River that drains a portion of the northeast area of the Pampean region, a vast grassy plain covering central Argentina,. The region is characterized by a warm climate with annual precipitations between 600 and 1200 mm, and an annual median temperature between 13 and 17 °C. Two stream reaches, 100 m long, separated by 5 km were chosen for the experiment. The stream reach located upstream (control) was kept intact while the downstream reach (treatment) was enriched with P. Location and physic-chemical conditions of both reaches are presented in Table 1.

2.2. Experimental Design and Field Methods

The enrichment was performed at the downstream reach by placing in-water bags with 1 kg of nutrients: 3/4 of a commercial fertilizer (Nitrofoska by Basf-Belgium, 12% of phosphorous as phosphate and 10% of nitrogen as nitrate) and 1/4 of Urea. They were located along three transects separated by 20 m at the beginning of treatment reach (4 bags per transect). The proportion of nutrient to add was calculated in order to maintain the stoichiometric relation of nutrients in water. All the nutrient content was released in two days (Table 2), so we replaced bags each 48/72 h. Dead standing leaves of *Typha latifolia* L. were harvested from both reaches in September 2008. Each plastic litterbag (20 × 15 cm, 10 mm mesh size) was filled with 12 pieces of *T. latifolia* 10 cm long pre-weighed (about 4 g per bag) and a small identification plastic sheet to allow the estimation of percentage of weight loss of each sample. The following week, groups of seven litterbags were placed in five sites separated by 20 m along each reach. Five replicates from each reach were collected after 2, 24, 52, 87, 112, 133 and 154 days. Water temperature and dissolved nutrient concentration were measured at each sampling date.

Table 1. Mean surface water characteristics during study period (September 2008–March 2009) (± standard deviation).

	Control	Treatment
Location (lat × long)	34°44′24″ S 59°06′27″ W	34°42′7″ S 59°04′36″ W
Water temp (°C)	22.5 (±7.9)	23.3 (±9.7)
pH	8.7 (±0.4)	8.3 (±0.1)
Conductivity (μS cm^{-1})	1224 (±39)	1700 (±47)
SRP (mg L^{-1})	0.06 (±0.04)	0.15 (±0.03)
NO_3 (mg L^{-1})	0.10 (±0.08)	0.10 (±0.01)

Table 2. Dynamics of nutrient (SRP and ammonia) concentration increase at the stream. 100% indicates a duplication at the background concentration (0.14 mg $P–PO_4^{3-}$ L^{-1} and 71 μg $N–NH_4^+$ L^{-1}).

	0 h	6 h	12 h	24 h	48 h	72 h	144 h
SRP (% increase)	0	100	71	50	30	1.5	0
Ammonia (% increase)	0	100	150	400	400	300	250

2.3. Laboratory Methods

At each sampling occasion, macroinvertebrates from each sample were sorted, identified and counted under a binocular magnifying glass, and functional groups were assigned according to Barbour *et al.* (1999). Litter samples were carefully rinsed with tap water to remove sediment and attached debris and then oven-dried at 60 °C to constant weight for biomass determination. Macroinvertebrate abundance was expressed as number of individuals per bag.

In five occasions (2, 24, 52, 87 and 133 days) exoenzymatic activities and P concentrations in *T. latifolia* were estimated. In order to quantify β-glucosidase (EC 3.2.1.21), cellobiohydrolase (EC 3.2.1.91) and alkaline phosphatase (EC 3.1.3.1–2) exoenzymatic activities, three fragments 5 cm long were separated from each sample before rinsing and then sonicated during three 3 min sessions separated by 1 min intervals, in 50 mL of tap water. The sonicated fragments were recovered to estimate their biomass and the water was used to measure enzymatic activities with fluorochrome-linked substrates (methylumbelliferyl [MUF]). The assays were done with a modification of the fluorogenic method (Romaní and Marxsen, 2002). Samples at a final concentration of 300 mmol L^{-1} of MUF (saturation concentration determined for these communities), MUF calibration solutions (0–100 μmol L^{-1}) and water controls were incubated for 1 h in darkness at stream temperature immediately after sampling. After incubation, Glycine buffer (pH 10.4) was added and fluorescence was measured at 365/455 nm (excitation/emission for MUF). Plant phosphorus content was determined by the ascorbic acid method (APHA, 1995) from 2 g dry-weighed *T. latifolia* grinded in a mortar, mufled and digested with hot HCl (25%). Exoenzymatic activities and *Typha* P content were expressed as nmol MUF h^{-1} and mg P per gram of plant, respectively.

Soluble reactive phosphorus (SRP), nitrate and ammonia concentrations in water were determined from 50 mL of stream water filtered through Glass Microfiber filters MGC immediately after their recollection (APHA, 1995). Nutrient concentrations were expressed as mg · L^{-1} of stream water.

2.4. Data Analyses

The changes in the undecomposed litter (X) as percentages of their initial quantity (X_0) at time T (days), were described following the equation of Olson (1963): $X = X_0 \cdot e^{-k \cdot T}$ where X is the mass at time T, X_0 is the initial mass, e the natural log constant and *k* the decomposition constant. Then, $k = -\log_e (X_T/X_0)/T$ and increasing values of *k* indicate increasing rates of litter mass loss.

As *T. latifolia* biomass was expected to vary both with treatment and time, we compared processing rates between reaches with an analysis of covariance (ANCOVA) of $\log_e (X_T/X_0)$ with Reach (two levels) as fixed factor and Time as the covariate (Bärlocher, 2005). Similarly, ANCOVA was used to compare phosphorus concentrations, exoenzymatic activities and invertebrate abundances, which also varied through the experiment, between treatments.

The relationship of *T. latifolia* P content and macroinvertebrate abundance with time was evaluated by the Pearson's correlation test.

All variables were checked for normality (Shapiro-Wilk, $P > 0.05$) and homogeneity of variances (Levene, $P > 0.05$) before parametric tests were performed. Variables that did not meet the assumption of normality were log transformed.

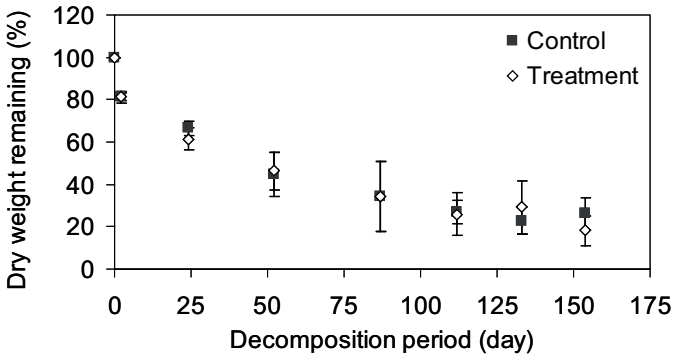

Figure 1. Percentage of *T. latifolia* decomposed throughout the experiment in the control and in the treatment reach. Bars indicate standard deviation.

3. Results

Artificial enrichment led to significant differences in phosphorus concentrations between control and treatment reaches (ANCOVA: $F_{1, 9} = 104.401$, $P < 0.001$).

In spite of dissolved P differences, decomposition rates were not significantly different between reaches (ANCOVA: $F_{1, 62} = 0.267$, $P = 0.601$) ($k_{\text{Control}} = 0.0087$ and $k_{\text{Treatment}} = 0.0097$ day^{-1}). *T. latifolia* lost about 77% of its initial weight during 154 days incubation in the stream (mean of relative weight lost for both reaches) (Fig. 1).

T. latifolia P content increased during the experiment in both reaches (Pearson's correlation: Control, $N = 25$, $R = 0.559$, $P = 0.004$ and Treatment, $N = 22$, $R = 0.806$, $P < 0.001$). A significant difference between reaches in *T. latifolia* P content was detected at the end of the experiment (ANOVA for day 133: $F_{1, 7} = 61.223$, $P < 0.001$) (Fig. 2).

Exoenzymatic activities varied seasonally and non-significant differences were found between reaches (ANCOVAs: β-glucosidase, $F_{1, 47} = 0.017$, $P = 0.898$; cellobiohydrolase, $F_{1, 47} = 1.732$, $P = 0.194$ and alkaline phosphatase, $F_{1, 47} = 0.003$, $P = 0.956$). The enzymes involved in polysaccharide degradation, β-glucosidase and cellobiohydrolase, presented a peak of activity between October 2008 and January 2009 (spring-summer in the Austral hemisphere). However, while the maximum activity of cellobiohydrolase was constant

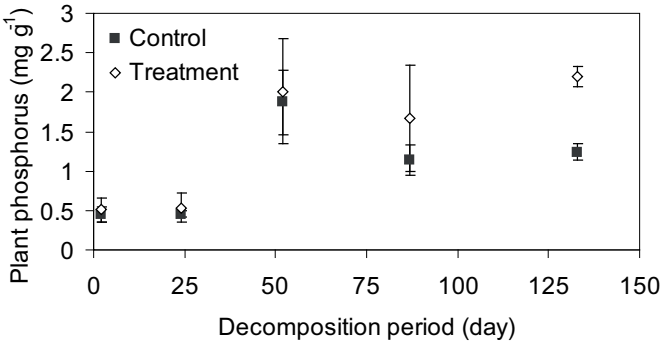

Figure 2. Mean phosphorus content of *T. latifolia* throughout the experiment in the control and in the treatment reach. Bars indicate standard deviation.

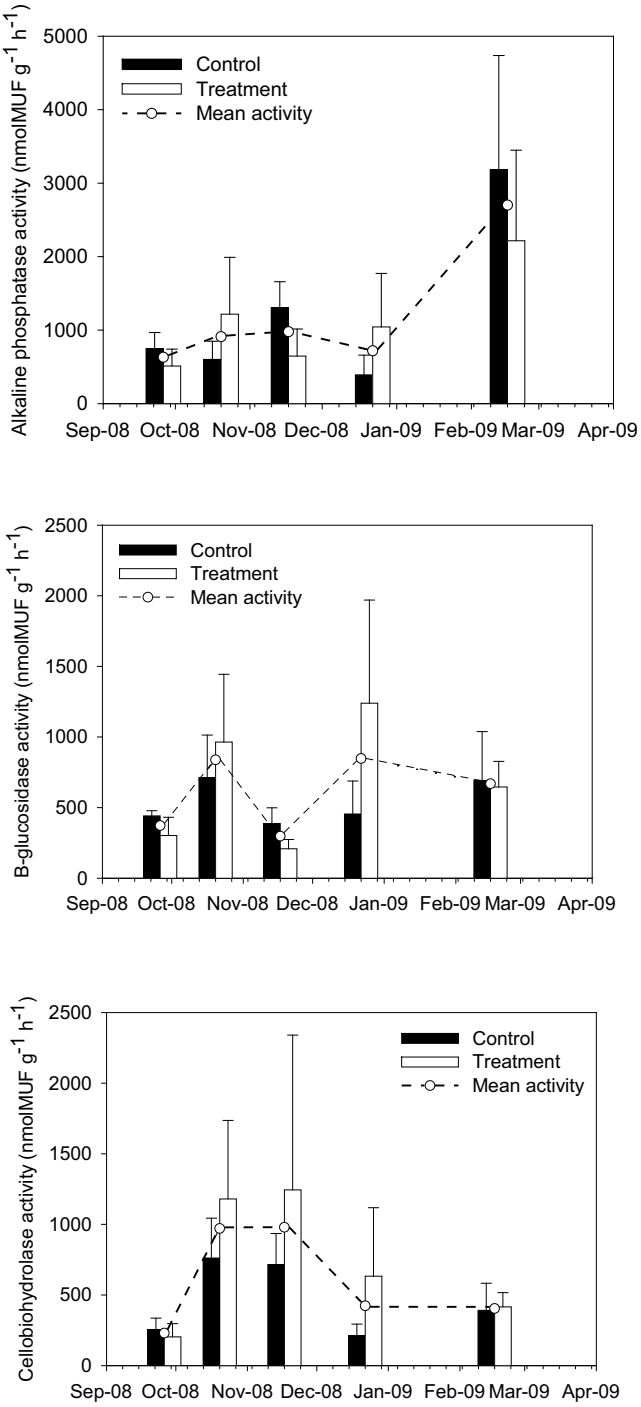

Figure 3. Mean enzymatic activities throughout the experiment in the control and in the treatment reaches. Bars indicate standard deviation.

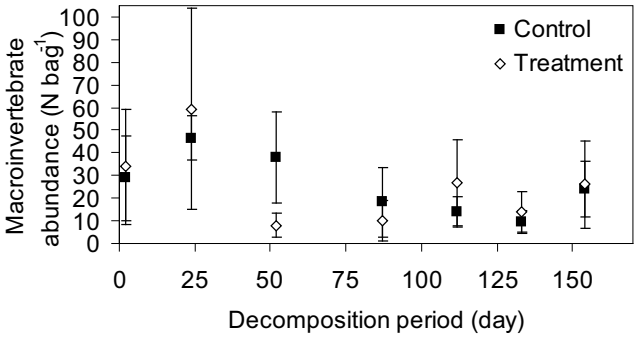

Figure 4. Mean macroinvertebrate abundances throughout the experiment in the control and in the treatment reaches. Bars indicate standard deviation.

between November and December, β-glucosidase suffered an important decrease in December and returned to maximum in January. Alkaline phosphatase presented an increase in activity in March 2009 (Fig. 3)

The macroinvertebrate community included 18 taxa, comprising 6 genera, 7 families, and 5 major groups. The community was dominated by Gastropoda (31% of total number of invertebrates in all sampling occasions; mean of the two reaches), Oligochaeta (17%), Ephemeroptera (11%), Hirudinea (11%) and Amphipoda (10%). The main functional feeding group theoretically involved in decomposition process, shredders, was represented by two genera of Decapoda (*Aegla* sp. and *Palaemonetes* sp.) which were always scarce (3%) and

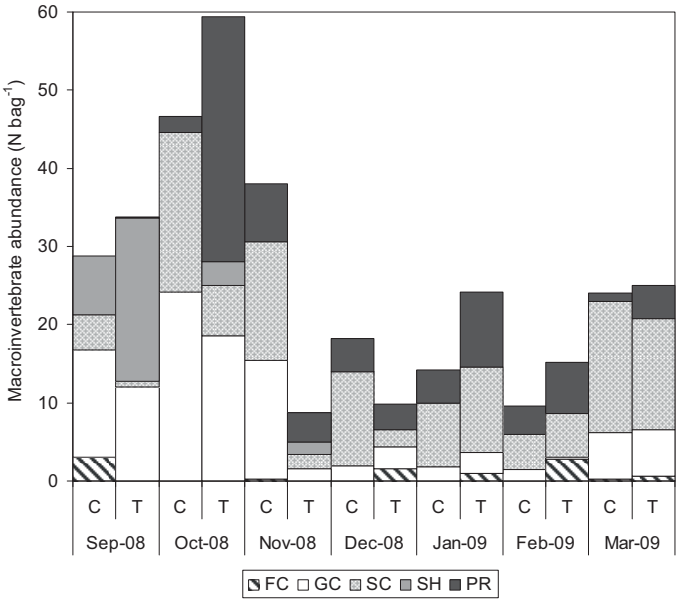

Figure 5. Mean macroinvertebrate abundances throughout the experiment in the control (C) and in the treatment (T) reaches, discriminated by functional feeding group. FC: filterer-collector, GC: gatherer-collector SC: scraper, SH: shredder, PR: predator (Barbour *et al.*, 1999).

completely absent after day 87. After a colonization peak by day 24, macroinvertebrate abundance decreased during the rest of the experiment (Pearson's correlation: $N = 65$, $R = -0.376$, $P = 0.002$) (Fig. 4). There were significant differences between reaches in abundance of predators (ANCOVA: $F_{1, 51} = 7.457$, $P = 0.009$), mainly Hirudinea, and scrapers (ANCOVA: $F_{1, 59} = 5.729$, $P = 0.02$), mainly Mollusca. However, shredder abundance was not significantly affected by nutrient enrichment (ANCOVA: $F_{1, 9} = 0.749$, $P = 0.409$) (Fig. 5).

4. Discussion

Decomposition rate in this Pampean stream was not affected by the amount of phosphorus dissolved in water, as only nonsignificant differences in decomposition rates were detected between control and enriched reaches. Although this result is opposite to the hypothesis which suggests that an increase in dissolved nutrients leads to an increase in decomposition rate (Elwood *et al.*, 1981; Peterson *et al.*, 1993; Sridhar and Bärlocher, 2000; Robinson and Gessner, 2000; Grattan and Suberkropp, 2001; Gulis and Suberkropp, 2003 a, b), it agrees with authors who have found a positive effect only of N but not of P concentration in water (Carpenter and Adams, 1979; Suberkropp and Chauvet, 1995) and those who have not found any effect of dissolved nutrients on the decomposition process (Abelho and Graça, 2006). In agreement with this, recent works suggest that decomposition rate may depend more on substrate conditions than on environmental conditions (Royer and Minshall, 2001; Kim and Rejmankova, 2004; Mille-Lindblom and Tranvik, 2003).

According to the lack of an effect of dissolved P on decomposition of *T. latifolia*, no differences in β-glucosidase and cellobiohydrolase activities, quantifiers of polysaccharides decomposition, or in alkaline phosphatase activity, quantifier of organic phosphorus compound decomposition, were detected between reaches along all the decomposition experiment. On the other hand, peaks of activity showed by β-glucosidase and cellobiohydrolase may be attributed to microorganism succession during decomposition. During the first days, coarse death material would have allowed fungal development which may have produced cellulolitic enzymes. Then, once more advanced decaying stages were reached an increase in the proportion of bacteria may have led to the peak in exoenzimatic activities typically released during bacteria growth (Gessner and Chauvet, 1997). This succession of microorganisms could be hard to detect due to the seasonal variations reported by other authors (Alvarez and Guerrero, 2000; Artigas Alejo, 2008), attributable to changes in stream physic (temperature, irradiation) and chemic (dissolved nutrients) characteristics.

With respect to the macroinvertebrates, their abundance decreased along the experiment, especially that of gatherer-collector, probably due to the decrease in *T. latifolia* biomass which provided food and refuge (González and Graça, 2005). In agreement with the lack of differences in decomposition rate and exoenzymatic activities, there were also no differences between reaches in macroinvertebrate abundance of the main group involved in decomposition process (shredders).

The decomposition rate of *T. latifolia* in this study, $k = 0.009$ day^{-1}, is high compared with most of the values reported for the genus (Table 3). However, a much higher value than this has been estimated (Nelson *et al.*, 1990). Since season and mesh size are able to affect decomposition rate (Alvarez and Becares, 2006), and the mesh size used in our study (10 mm) allowed the entrance of all the macroinvertebrate groups present in the stream, we think that our decomposition rate may be a good estimation for *T. latifolia* under natural conditions during spring-summer in a Pampean stream.

Previous work has shown that in Pampean streams the autotrophic component of biofilms (epiphyton) can increase its nutrient uptake and growth as a result of artificial enrichment under laboratory conditions (Giorgi, 1995). If we attribute the increment in P concentra-

Table 3. Decomposition rates of *Typha* spp. available in literature.

Species	P detritus (% DW)	P water (mg L^{-1})	Decay rate (day^{-1})	Reference
Typha domingensis	% P = 0.012	–	0.00099	Davis, 1991
Typha domingensis	% P = 0.014	–	0.0010	Davis, 1991
Typha glauca	% P = 0.025	–	0.0011	Neeley and Davis, 1985
Typha domingensis	% P = 0.028	–	0.0021	Davis, 1991
Typha glauca	% P = 0.050	–	0.0011	Neeley and Davis, 1985
Typha glauca	% P = 0.050	–	0.0104	Nelson *et al.*, 1990
Typha glauca	% P = 0.092	–	0.0012	Van Der Valk *et al.*, 1991
Typha glauca	% P = 0.108	–	0.0012	Van Der Valk *et al.*, 1991
Typha glauca	% P = 0.290	–	0.0235	Nelson *et al.*, 1990
Typha spp. (23 °C)	–	Total P = 0.080–0.230	0.004	Bruquetas and Neiff, 1991
Typha spp. (26 °C)	–	PRS = 0.000633	0.045	Ruppel *et al.*, 2004
Typha angustifolia (17 °C)	–	Total P = 0.09	0.0098	Jaques and Pinto, 1997
Typha angustifolia (18 °C)	–	Total P = 0.16	0.0031	Jaques and Pinto, 1997
Typha latifolia (23 °C)	% P = 0.044	Total P = 0.126 PRS = 0.055	0.0087	this study
Typha latifolia (23 °C)	% P = 0.051	Total P = 0.234 PRS = 0.152	0.0097	this study
Typha glauca	–	–	0.0016	Neeley and Davis, 1985
Typha sp.	–	–	0.001	Findlay *et al.*, 1990
Typha latifolia (5 °C)	–	–	0.0014–0.0026	Alvarez and Becares, 2006
Typha latifolia (20 °C)	–	–	0.0043–0.0052	Alvarez and Becares, 2006

tion in *T. latifolia* along the experiment to immobilization by microorganisms (Qualls and Richardson, 2000; Abelho and Graça, 2006), the significant difference found between reaches at the end of the experiment indicates that decomposers would also be capable of increasing their nutrient absorption as a result of nutrient enrichment.

The lack of a clear response of decomposition rate and exoenzymatic activities to enrichment, despite phosphorus immobilization in decomposing material, may have at least three explanations. First, the experiment may have not been long enough to detect an effect on microbial growth and hence on the amount of exoenzymes released by decomposing organisms. However, this is unlikely as some decomposition experiments have detected a peak in aquatic fungi biomass after only 60 days (Gessner *et al.*, 2007) and our decomposition period extended for about five months. Second, the nutrients may have been used for other processes rather than for growth. For instance, Abelho and Graça (2006) found in a nutrient-rich stream that it was not fungal growth but reproduction that was limited by dissolved phosphorus concentration. Finally, it is also possible that, similarly to Royer and Minshall (2001) the stream biota did not have a phosphorus limitation, and that the observed phosphorus absorption was a response similar to the luxury uptake found in algae and plants (Stevenson and Stoermer, 1982; Kröger *et al.*, 2007).

In conclusion, although artificial enrichment led to significant differences in SRP and decomposing material P content between treatments, these differences did not result in a higher decomposition rate, exoenzymatic activity or abundance of macroinvertebrates. Hence, in the naturally nutrient-rich Pampean streams decomposing organisms may be more likely to store or use additional nutrients for processes like reproduction instead of using them for growth.

5. Acknowledgements

We thank to BBVA foundation for support this study, to Anna Romani by the detailed explanation about the application of the enzymatic techniques and to Arturo Elosegi for his valuable discussion.

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Manuscript submitted February 12th, 2011; revised April 7th, 2011; accepted April 13th, 2011

