Leaf litter decomposition (and invertebrate colonization) in alpine environments above the tree line: an experimental study

(Article begins on next page)

Tiziano Bo [1], Massimo Cammarata [1], Manuel Jesus López-Rodríguez [2], Josè Manuel Tierno de Figueroa [3] and Stefano Fenoglio [1*]

[1] Dipartimento di Scienze di Scienze ed Innovazione Tecnologica, Università del Piemonte Orientale, Via Teresa Michel 11, 15121, Alessandria, Italy (fenoglio@unipmn.it).
[2] Departamento de Ecología. Facultad de Ciencias, Universidad de Granada, 18071, Granada, Spain.
[3] Departamento de Zoología, Facultad de Ciencias, Universidad de Granada, 18071, Granada, Spain.

* corresponding author: e-mail: fenoglio@unipmn.it

LEAF LITTER DECOMPOSITION(AND INVERTEBRATE COLONIZATION) IN ALPINE ENVIRONMENTS ABOVE THE TREE LINE: AN EXPERIMENTAL STUDY

ABSTRACT

Recent studies suggested a general warming trend in the Alps, resulting in a significant migration of forests to altitudes higher than the usual, regional tree line. As a consequence, some headwater streams will likely receive more allochthonous organic matter. For this reason, the dynamics of decomposition of terrestrial leaf detritus in stream reaches that naturally lacked this resource represents a subject of considerable interest, on which no information is currently available. The aim of this study was to analyse breakdown and macroinvertebrate colonisation of leaf bags in an Alpine headwater stream above the tree line. Results of this study indicate that decomposition of terrestrial leaves in a lotic alpine environment above the tree line takes place through a process similar to what happens at lower altitudes, but with some differences. The reduced rate of decomposition observed may be due to lower temperatures. At lower altitudes, tree cover provides a supply of organic material sufficient to support a rich guild of shredders. This study demonstrates that also above tree line, where communities are dominated by scrapers, an important part of the benthic community take part in the decomposition process of leaves. We can conclude that streams above the tree line, while hosting invertebrate communities dominated by rhithrophilous organisms that feed mostly on biofilm, also harbour a rich population of opportunist invertebrates. It seems that, in the case of expected temperature increase at higher altitudes , terrestrial organic detritus may be actively degraded by lotic benthic communities.

KEY WORDS: Climate change, Alps, Stream ecology, detritus processing, macroinvertebrates, Po river.

1. INTRODUCTION

The role of allochthonous detritus in lotic food webs has been studied for a long time (Odum 1956, Kaushik and Hynes 1971, Petersen and Cummins 1974). The understanding of the importance of connections across terrestrial and aquatic boundaries was a harbinger for major developments in stream ecology, with innovative and different schools of research that led to the formulation of theories such as the River Continuum Concept (Vannote *et al.* 1980). Tank *et al.* (2010) summarized results of studies regarding decomposition processes and characteristics of allochthonous organic matter budgets in lotic ecosystems. Methods used in decomposition studies have been reviewed by Boulton and Boon (1991) and more recently by Graça *et al.* (2005). The processing of leaf litter in streams involves a series of well documented steps: leaching, physical break up, microbial conditioning, macroinvertebrates colonisation and fragmentation (Woodward . 2012). Leaf breakdown is commonly measured as mass loss over time and is usually assessed using leaf bag decomposition studies. The rate of leaf litter breakdown depends on external factors, such as temperature and the chemical characteristics of water (Pascoal *et al.* 2003, Fenoglio *et al.* 2006), microbial activity (such as bacteria - Maamri *et al.* 1998, aquatic hyphomycetes – Gessner and Chauvet 1997) and macroinvertebrate activity (Graça *et al.* 2001), and internal factors, such as leaves characteristics (Swan and Palmer 2004, Riipinen *et al.* 2009). Recently, interest in this topic has grown further because of its application to environmental monitoring. Several studies suggest using leaf breakdown rates as indicator of anthropogenic disturbance on stream ecosystem integrity (e.g., Webster and Benfield 1986, Niyogi *et al.* 2001, Young *et al.* 2008). Information on leaf breakdown dynamics is abundant for temperate, Mediterranean, (Casas and Gessner 1999, Peralta-Maraver *et al.* 2011), and tropical lotic environments (Shieh *et al.* 2007, Mathuriau and Chauvet 2002).

Benthic macroinvertebrates play a main role in lotic food webs (Fleituch and Amirowicz 2005), and particularly in the leaf breakdown process (Fleituch 2013). According to Merritt and Cummins (1996) and Tachet et al. (2002), these organisms can be classified into five Functional Feeding Groups: scrapers, which consume algae and associated material; shredders, which consume leaf litter or other coarse particulate organic matter (CPOM); collector-gatherers, which collect fine particulate organic matter (FPOM) from the stream bottom; collectors-filterers, which collect FPOM from the water column; and predators, which feed on other invertebrates. Leaf detritus represents an important resource for invertebrate shredders, that play a substantial role in the decomposition process of this allochthonous input (Allan 1995; Hieber and Gessner 2002; Fleituch 2013). Through the production of faecal pellets and orts (fine fragments shredded from leaves but not ingested), shredders convert CPOM into FPOM, which is then distributed downstream and ingested by many other consumers (collector-gatherers and filterers; Pretty *et al*. 2005). However, most studies published so far were performed on forested streams, or on rivers that flow into basins with at least some extent of tree cover (but see Hladyz S. *et al.* 2011).

Interestingly, very little information is available about allocthonous detritus processing in high-altitude Alpine streams (but see Robinson and Gessner 2000, Gessner and Robinson 2003). Because of harsh climatic and edaphic characteristics, alpine catchments encompass particular types of vegetation cover, characterized by scarce biomass above tree line. The tree line is not usually distinct but very variable and separates areas with different growth limitation factors for trees (Körner 1998). Above the tree line, alpine streams usually lack significant riparian inputs of organic matter that are typical of streams of temperate areas (Ward 1994). In recent years, some studies suggested that a significant increase in forest cover can be found beyond the usual, regional tree line (Grace *et al.* 2002, Parolo and Rossi 2008, Didion *et al.* 2011). This upward shift can be attributed to climate change, and it is likely to further if the climate continues to warm (Gehrig-Fasel *et al.* 2007). With the hypothesized vertical migrations of trees, some headwater streams are likely to receive more allocthonous organic matter. For this reason, the dynamic of decomposition of terrestrial leaf detritus in stream reaches that naturally lacked this resource is a subject of considerable interest, on which virtually no information is currently available. The aim of the present study is to analyse leaf breakdown and macroinvertebrate colonisation in an alpine, headwater stream above the tree line. Our hypothesis is that the decomposition and macroinvertebrate colonisation of leaves could have similar patterns than what occurs in lowland, forested Alpine stream reaches.

2. MATERIAL AND METHODS

The study was carried out in the Po River at Crissolo, Pian della Regina (1750 m a.s.l., UTM 357750-4951547, NW Italy - Fig. 1). The Po River is the longest Italian lotic system (652 km) and rises from a spring under the northwest face of the Monviso, in the Cottian Alps. In our study site, which is located 2.5 km downstream from the source, the Po River is a typical 2nd order, high gradient mountain stream. Several lakes of glacial origin are present in the highest part of the catchment, with no direct surface connections with the Po River. In the study site, the stream crosses a plain of glacial origin, characterized by the presence of extensive alpine prairie, large boulders, and very few and scattered European larch (*Larix decidua* Mill). In this site, the channel width was about 4-6 m, with a mean depth of 40-60 cm, and the prevalent stream substrate consisted of boulders and cobbles. The climate is temperate-alpine, with springtime high discharge caused by snowmelt. This research was carried out between October 2009 and May 2010. Recently fallen leaves of Chestnut (*Castanea sativa* Miller) were collected from the ground in a downstream area, at 1000 m a.s.l. in the same valley. Leaves were transported to the laboratory and dried for 15 days at room temperature. In total, e assembled 70 bags of leaves (dry mass of each bag: 5.11 g mean ± 0.11 SD), tied together with a nylon mesh (1 cm mesh size), to allow macroinvertebrate colonisation. Bags were introduced in the stream on October 29, 2009. Bags were tied to boulders and randomly submersed in a 200 m homogeneous stream reach. Ten leaf bags on each sampling occasion with one month interval were randomly collected until May 2010, when no detritus was found in the nylon meshes. After removal, bags were placed separately in plastic bags with stream water and then immediately transported to the laboratory. Macroinvertebrates were directly collected with tweezers and preserved in 70 % ethanol. All organisms counted were identified down to the genus level, except for Chironomidae, Simuliidae, and early instars of some Trichoptera and Diptera, which were identified to the family level. Each taxon was also assigned to a Functional Feeding Group (FFG – Merritt and Cummins, 1996; Tachet et al. 2002). To compare the abundances of invertebrates that colonized the leaf bags with the natural densities of macroinvertebrates in the stream, eight Surber were randomly collected in each sampling date, accounting for a total area of 0.5 m^2. Invertebrates from these Surbers (250 µm mesh), was mixed and introduced into a single plastic jar with 70 % ethanol and then transported to the laboratory. Leaves were oven dried at 105 °C until a constant mass was reached in order to determine the remaining mass. Leaf mass loss was calculated and using percentage remaining mass (%R) after Petersen and Cummins (1974): $\%R = W_{(t)}/W_{(0)} \times 100$, were $W_{(0)}$ is the initial mass and $W_{(t)}$ represents the amount of material remaining after time *t*. We also calculated the mean daily water temperature for each day that leaf bags was in the stream, and then we summed means to get degree-day for each leaf bag.

During the study period, water temperatures were measured hourly with a Datalogger (HOBO® Water Temp Pro, 0.01 ºC accuracy – Fig. 2). Other physical-chemical parameters were measured on each sampling date using Eijkelkamp 13.14 and 18.28 portable instruments (Table 1).

Statistical analyses of: the density (N), taxonomic richness (S), and FFG composition of macroinvertebrates were performed using ANOVAs and Bartlett Chi-square tests on log (N, S) or arcsin-transformed (FFG) data. All the statistical analyses were calculated using STATISTICA v.7.1 software (StatSoft 2005).

3. RESULTS

3.1.Mass loss

An exponential mass loss over time was observed in the leaf bags. The decrease was rapid during the first 30 days and the mean remaining mass at the first removal date was 66.7 % of the initial mass (Fig. 3a). Mass loss of leaves was approximated by an exponential decay model, so that decomposition rate can be modelled as follows: $Wt=152.7e^{-0.016t}$. The decomposition process was quite uniform also considering the percentage of leaf material lost per degree-day (Fig. 3b).

3.2.Macroinvertebrate colonization

A total of 3204 macroinvertebrates belonging to 29 taxa were collected in the 70 litter bags, and 18964 macroinvertebrates from seven benthic samples, belonging to 41 taxa. The abundance of colonizing macroinvertebrates in the leaf bags was significantly affected by time (ANOVA $F_{6,63} = 24.8$, $P < 0.001$): mean number of organisms increased from 44.2 ± 6.83 SE individual per /bag in the first removal date to 89.8 ± 18.5 SE individuals/bag in the third date and decreased linearly to a value of 9.10 ± 2.14 SE individual per bag in the last removal date. Taxa richness (S) varied significantly over time (ANOVA $F_{6,63} = 10.9$, $P < 0.001$). S remained almost constant in the first four removal dates (about seven taxa), and then decreased, reaching the lowest values in the last two dates (Fig. 4). S was significantly related to remaining leaf mass (Bartlett Chi-square: 5.51 df=1, $P < 0.05$), but not to total invertebrate number (N - Bartlett Chi-square: 2.71 df=1 $P = 0.09$).

The macroinvertebrate assemblages colonising leaf bags and stream substrate were also classified into Functional Feeding Groups (FFG- Figure 5). Functional composition of macroinvertebrate assemblages between natural riverbed and leaf bags was compared, and differences were detected. Collector-gatherers were more abundant in the leaf bags than in the substrate (38.9 % and 16.4 % respectively; t-test = 2.31, $P<0.05$). The number of shredders was also higher in leaf-bags than in the substrate, but this difference was not statistically significant (t-test = 2.05, $P = 0.06$). The greatest variation was recorded for the scrapers group (t-test = 5.47, $P < 0.001$). Scrapers were scarce in the leaf bags and more abundant in the stream bed (1.9 % and 36.4 % of the total community, respectively). Predators represented 13.0 % of collected invertebrates in the leaf bags and 11.5 % in the substrate, with no significant differences (t-test = 0.68, $P = 0.51$). Filterers was the least frequent FFG in both assemblages (0.6 % in the leaves and 1.81 % in the substratum), with no significant difference in percentage composition (t-test = -1.07, $P = 0.30$).

4. DISCUSSION

In this study, we analysed the decomposition of terrestrial tree leaves in an Alpine stream. Our initial hypothesis was that, above the tree line, decomposition and macroinvertebrate colonisation of leaf litter could have similar patterns than at lower altitudes, in forested areas. Results suggest that aquatic decomposition of terrestrial leaves above the tree line takes place through a process generally comparable to what happens at lower altitudes, with some differences in the breakdown rate and in the composition of colonising assemblages. The results from this study indicate that macroinvertebrate communities in Alpine prairie streams can promptly adapt to consume unusual food sources.

In our study site, Chestnut leaf-bags exhibited an exponential mass loss over time and were colonised by part of the macrobenthic community present in this stream reach. Mass loss was faster during the first month, probably because of the leaching of soluble materials (Tank et al. 2010). Breakdown of *C. sativa* leaves was lower than what reported in similar studies at lower altitudes (Fenoglio *et al.* 2006, Canhoto and Graça 1996). The decomposition rate in this study was lower than what reported for Chestnut leaf-bags in the Erro Creek, a stream located in the same region but at lower altitude (about 350 m a.s.l. – Fenoglio *et al.* 2006). Because the study on the Erro Creek lasted for less time, we can only compare the first two removal dates. After two months spent in the streambed, the leaf bags in the River Po showed a residual mass of 61.9 % while those in the river Erro had a residual mass of 47.8 %. Despite this difference, the decay process was exponential, following a profile similar to that reported in other studies realized in forested environments (see review in Tank *et al.* 2010).

Leaf bags were promptly colonized by diverse and rich assemblages of benthic macroinvertebrates that use leaves as a source of food and/or a shelter and refuge. There were some significant differences in the functional composition of macroinvertebrate assemblages colonizing the natural riverbed and the leaf bags. In particular, while scrapers represented the most important FFG in the substrate, their abundance was minimal in the leaf bags (1.93 % of total invertebrates). Shredders were an important component in both assemblages, and represented about half of the assemblage that colonized the leaf bags. The relative minor abundance of shredders at the first removal date is probably due to the incomplete microbial conditioning of leaves. Collector-gatherers constituted approximately the 40 % of colonising assemblages of leaf bags These may act as traps for fine particulate organic matter, where collectors can utilize both leaf fragments and organic particles trapped in the bags. We can also assume that leaf bags do not represent just a trophic resource, but constitute a particular micro-environment, which harbour organisms that prefer moderate current velocity, do not feed on biofilm, and preferentially consume coarse vegetal material. In the last removal date (Fig. 5) we detected an increase in the abundance of Filterers, probably due to the increased amount of FPOM detaching from the bags

The process of decomposition of terrestrial leaves is comparable between a lotic alpine environment above the tree line and in similar lotic systems at lower altitude. It is known that temperature is one of the parameters that mostly influences the rate of biotic decomposition of organic detritus (Webster and Benfield 1986), and the reduced rate of decomposition that we observed may be, in fact, due to low temperatures.

In this study, we demonstrated that above tree line, where communities are dominated by invertebrate scrapers, an important part of the benthic community is also involved in the decomposition process of leaves. Some recent studies have indicated that also lotic systems draining catchments lacking a significant number of trees, such as prairie or intensive-agricultural streams, can receive significant amounts of organic matter from the surrounding riparian zone (Stagliano and Whiles 2002, Hagen *et al.* 2006). Mountain prairie streams above the tree line, while hosting invertebrate communities dominated by rhithrophilous organisms that feed mostly on biofilm, also harbour a rich population of opportunist invertebrates that can consume coarse particulate organic matter. These organisms usually feed on small sized particles of allochthonous detritus (grass fragments, small herbaceous plant parts, flowers, seeds) but, in presence of a conspicuous input of terrestrial leaves, are able to actively colonize and degrade this resource.

It is well known that mountain ecosystems are particularly sensitive to alterations in climatic conditions (Beniston 2006). In the event of an altitudinal rise of the thermal boundary for tree growth, due to climate change in the Alps, it is likely that the organic detritus produced by trees could be actively degraded by lotic benthic communities.

Acknowledgements

This study was supported by Fondazione Cassa di Risparmio di Alessandria CRAL grants. We are also grateful to the Editor, referees and to M. Pessino and T.A. Catanach (University of Illinois) for useful comments and English revision.

5. REFERENCES

Allan J.D. 1995 - Stream ecology. Structure and function of running waters. - Chapman & Hall, London, U.K., 388 pp.

Beniston M. 2006 - Mountain weather and climate: a general overview and a focus on climatic change in the Alps - Hydrobiologia 562: 3-16.

Boulton A.J., Boon P.I. 1991 - A review of methodology used to measure leaf litter decomposition in lotic environments: time to turn over an old leaf? - Aust. J. Mar. Fresh. Res. 42: 1-43.

Canhoto C., Graça M.A.S. 1996 - Decomposition of *Eucalyptus globulus* leaves and three native leaf species (*Alnus glutinosa*, *Castanea sativa* and *Quercus faginea*) in a Portuguese low order stream - Hydrobiologia 333: 79-85.

Casas J.J., Gessner M.O. 1999 - Leaf litter breakdown in a Mediterranean stream characterised by travertine precipitation - Freshwat. Biol. 41: 781-793.

Didion M., Kupferschmid A.D., Wolf A., Bugmann H. 2011 - Ungulate herbivory modifies the effects of climate change on mountain forests - Climatic Change 109: 647-669.

Fenoglio S., Bo T., Cucco M., Malacarne G. 2006 - Leaf breakdown patterns in a NW Italian stream: effect of leaf type, environmental conditions and patch size - Biologia Bratislava 61: 555-563.

Fleituch T., Amirowicz A. 2005 - Stream habitats, benthic macroinvertebrates, lotic fish and their relationships: a multi-scale approach - Pol. J. Ecol. 53: 81-95.

Fleituch T. 2013 – Effects of nutrient enrichment and activity of invertebrate shredders on leaf litter breakdown in low order streams .- Int. Rev. Hydrobiol.98:191-198

Gehrig-Fasel J., Guisan A., Zimmermann N.E. 2007 - Tree line shifts in the Swiss Alps: climate change or land abandonment? - J. Veg. Sci. 18: 571-582.

Gessner M., Chauvet E. 1997 - Growth and production of aquatic hyphomycetes in decomposing leaf litter - Limnol. Oceanogr. 42: 496-505.

Gessner M.O., Robinson C.T. 2003 - Aquatic hyphomycetes in alpine streams (In: Ecology of a glacial flood plain, Eds: J.V. Ward, U. Uehlinger) - Dordrecht, Kluwer, pp. 123-136.

Graça M.A.S., Barlocher F., Gessner M.O. 2005 - Methods to study litter decomposition: a practical guide - Springer, Dordrecht, The Netherlands, 329 pp.

Graça M.A.S., Ferreira R.C.F., Coimbra C.N. 2001 - Litter processing along a stream gradient: the role of invertebrates and decomposers -J. N. Am. Benthol. Soc. 20: 408-420.

Grace J., Berninger F., Nagy L. 2002 - Impacts of climate change on the tree line – Ann. Bot. London 90: 537-544.

Hagen E.M., Webster J.R., Benfield E.F. 2006 - Are leaf breakdown rates a useful measure of stream integrity along an agricultural land use gradient? - J. N. Am. Benthol. Soc. 25: 330-343.

Hieber M., Gessner M.O. 2002 - Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. - Ecology 83: 1026-1038.

Hladyz S. et al. 2011. Stream ecosystem functioning in an agricultural landscape: the importance of terrestrial-aquatic linkages. - Adv. Ecol. Res. 44: 211-276.

Kaushik N.K., Hynes H.B.N. 1971 - Fate of dead leaves that fall into streams – Arch. Hydrobiol. 68: 465-515.

Körner C. 1998 - A re-assessment of high elevation treeline positions and their explanation - Oecologia 115: 445-459.

LeRoy C.J., Whitham T.G., Wooley S.C., Marks J.C. 2007 - Within-species variation in foliar chemistry influences leaf-litter decomposition in a Utah river – J. N. Am. Benthol. Soc. 26: 426-438.

Maamri A., Chauvet E., Chergui H., Gourbiere F., Pattee E. 1998 - Microbial dynamics on decaying leaves in a temporary Moroccan river. I – Bacteria – Arch. Hydrobiol. 144: 157-175.

Mathuriau C., Chauvet E. 2002 - Breakdown of leaf litter in a neotropical stream - J. N. Am. Benthol. Soc. 21: 384-396.

Merritt R.W., Cummins K.W. 1996 - An Introduction to the aquatic insects of North America. Kendall Hunt, Dubuque, U.S.A., 862 pp.

Niyogi D.K., Lewis W.M., Mcknight D.M. 2001- Litter breakdown in mountain streams affected by mine drainage: biotic mediation of abiotic controls – Ecol. Appl. 11: 506-516.

Odum H.T. 1956 - Primary production in flowing waters – Limnol. Oceanogr. 1: 102-117.

Parolo G., Rossi G. 2008 - Upward migration of vascular plants following a climate warming trend in the Alps - Basic Appl. Ecol. 9: 100-107.

Pascoal C., Pinho M., Cassio F., Gomes P. 2003 - Assessing structural and functional ecosystem condition using leaf breakdown: a study in a polluted river - Freshwat. Biol. 48: 2033-2044.

Peralta-Maraver I., López-Rodríguez M.J., Fenoglio S., Bo T., Luzón-Ortega J.M., Tierno de Figueroa J.M. 2011 - Macroinvertebrate colonization of two different tree species leaf bags (native vs. introduced) in a Mediterranean stream – J. Freshwater Ecol. 26: 495-505.

Petersen R.C., Cummins K.W. 1974 - Leaf processing in a woodland stream – Freshwat. Biol. 4: 343-368.

Pretty J.L., Giberson D.J., Dobson M. - 2005. Resource dynamics and detritivore production in an acid stream - Freshwater Biol. 50: 578–591.

Riipinen M.P., Fleituch T., Hladyz S., Woodward G., Giller P., Dobson M. - 2009. Invertebrate community structure and ecosystem functioning in European conifer plantation streams - Freshwater Biol. 55: 346-359.

Robinson C.T., Gessner M.O. 2000 - Leaf breakdown in an alpine spring brook - Verh. Internat. Verein. Theor. Angew. Limnol. 27: 744-747.

Shieh S.H., Hsu C.B., Wang C.P., Yang P.H. 2007 - Leaf breakdown in a subtropical low order stream riffle and its associated macroinvertebrates – Zool. Stud. 46: 609-621.

Stagliano D.M., Whiles M.R. 2002 - Macroinvertebrate production and trophic structure in a tallgrass prairie headwater stream – J. N. Am. Benthol. Soc. 21: 97-113.

StatSoft, Inc. 2005: STATISTICA (data analysis software system), version 7.1. (www.statsoft.com, accessed July 2011).

Swan C.M., Palmer M.A. 2004 - Leaf diversity alters litter breakdown in a Piedmont stream – J. N. Am. Benthol. Soc. 23: 15-28.

Tachet H., Bournaud M., Richoux M. 2002 - Introduction à l'étude des macroinvertébrés d'eau douce : (systématique élémentaire et aperçu écologique) - Université Lyon 1, Villeurbanne, France, 155 pp.

Tank J.L., Rosi-Marshall E.J., Griffiths N.A., Entrekin S.A., Stephen M.L . 2010 - A review of allochthonous organic matter dynamics and metabolism in streams – J. N. Am. Benthol. Soc. 29: 118-114.

Vannote R.L., Minshall G.W., Cummins L.W., Sedell J.R., Cushing C.E. 1980 - The river continuum concept . Can. J. Fish. Aquat. Sci. 37: 130-137.

Ward J.V. 1994 - Ecology of alpine streams – Freshwat. Biol. 32: 277-294.

Webster J.R., Benfield E.F. 1986 - Vascular plant breakdown in freshwater ecosystems - Annu. Rev. Ecol. Syst. 17: 567-594.

Woodward G., Gessner M.O., Giller P.S., Gulis V., Hladyz S., Lecerf A., Malmqvist B., McKie B. G., Tiegs S. D., Cariss H., Dobson M., Elosegi A., Ferreira V., Graça M.A. S., Fleituch T., Lacoursière J.O., Nistorescu M., Pozo J., Risnoveanu G., Schindler M., Vadineanu A., Vought L.B., Chauvet E. 2012 - Continental-scale effects of nutrient pollution on stream ecosystem functioning - Science 336: 1438-1440.

Young R.G., Matthaei C.D., Townsend C.R. 2008 - Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health - J. N. Am. Benthol. Soc. 27: 605-625.

Parameter	**Values**
Conductivity (μS/cm)	95 ± 0.16
C.O.D. (mg/l)	< 5.0
B.O.D. (mg/l)	< 2.0
Total P (mg/l)	< 0.05
NH_4^+ (mg/l)	< 0.05
O_2 (mg/l)	11.1 ± 0.92
O_2 (%)	90.4 ± 8.45
Flow speed (m/s)	0.43 ± 0.15
Mean temperature (°C)	2.89 ± 1.36
pH	7.56 ± 0.09

Table 1 - Mean physical-chemical parameters in the Po River during the study period (mean ± SD).

Fig. 1 - Location of the study site in the Po River (NW Italy).

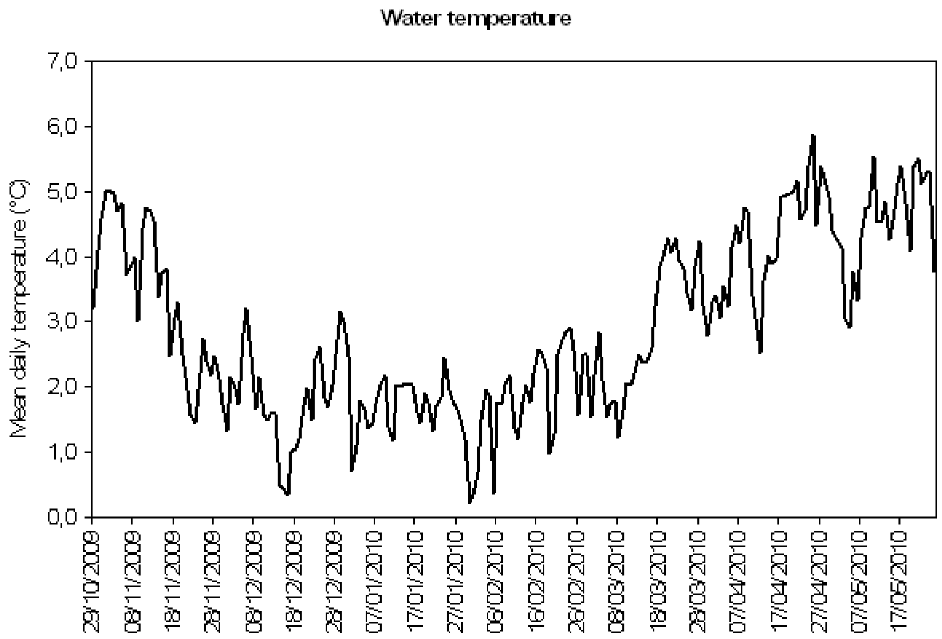

Fig. 2 - Water temperature during the study period.

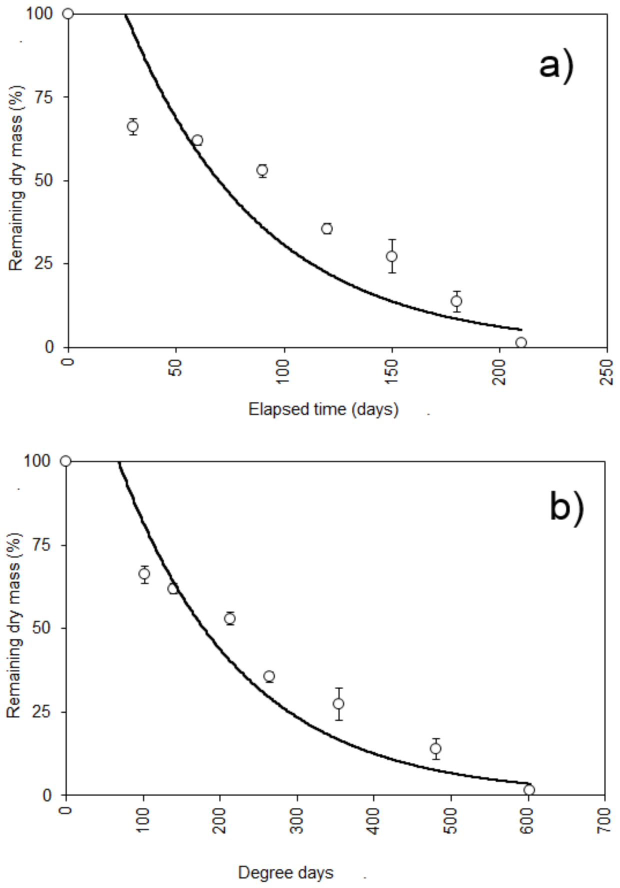

Fig. 3 - Remaining mass (percentage of original dry mass, mean ± se) of leaf bags in the seven removal dates (a) and compared with degree-day accumulation (b).

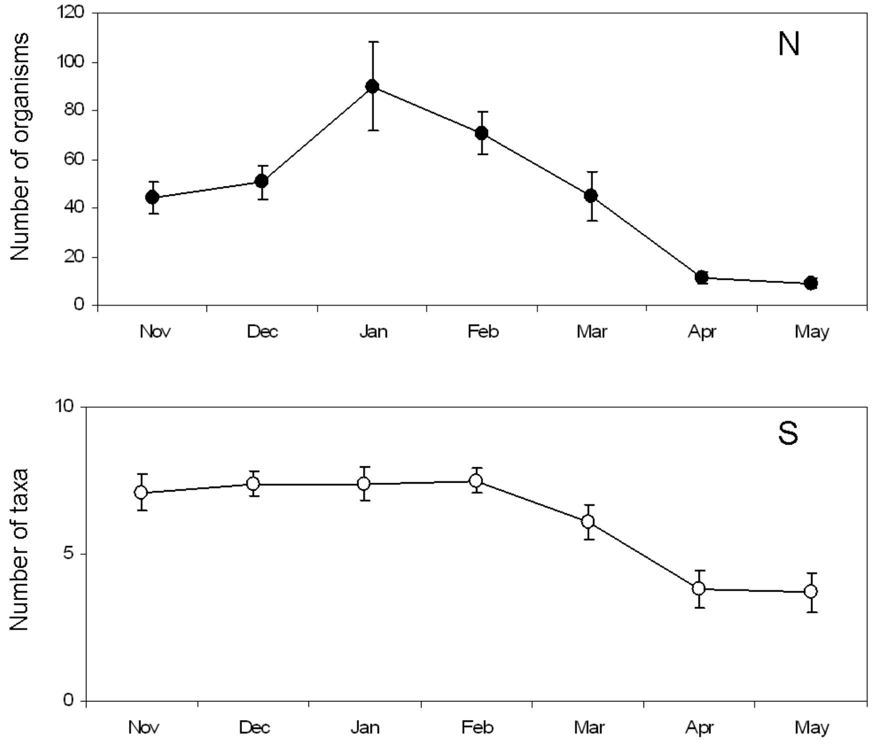

Fig. 4 - Variation in the macroinvertebrate number and taxa in leaf bags during the study period (N = number of invertebrates; S = taxon richness).

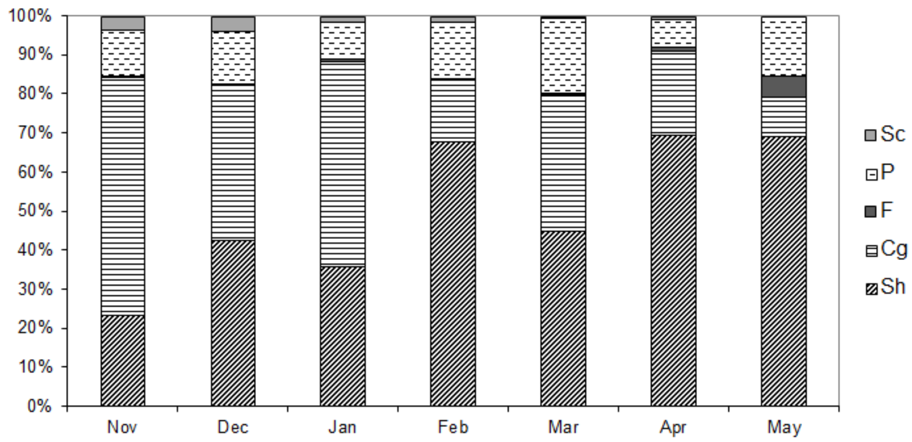

Fig. 5 – Relative importance of the five Functional Feeding Groups in the bags during the study period (Sc = Scrapers; P = Predators; F = Filterers; Cg = Collectors-Gatherers; Sh = Shredders).