ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., Suppl. 7, 2014: 197-202

Decomposition of Willow Leaf Litter in an Oxbow Lake of the Danube River at Gemenc, Hungary

Edit Ágoston-Szabó*, Károly Schöll, Anita Kiss, Árpád Berczik, Mária Dinka

MTA Centre for Ecological Research, Hungarian Academy of Sciences, Danube Research Institute, Alkotmány u. 2-4, 2163 Vácrátót, Hungary; *Email: agoston-szabo.edit@okologia.mta.hu

Abstract: The decomposition dynamics of willow (*Salix alba* L.) leaf litter was studied in Nyéki-Holt-Duna/ Nyéki Danube Oxbow Lake, using litter bag technique. using litter bag technique. We determined the dry mass; ash free dry mass (AFDM) and litter chemical composition as C, N, P, S remaining. In addition, we studied microbiological parameters, such as the litter associated fungal biomass by ergosterol concentration and the potential microbial respiration by electron transport system (ETS) activity. The loss of mass and the release of nutrients followed a simple negative exponential pattern during the 140 day of the decomposition period. By the end of the decomposition 31% AFDM, 33% C, 50% N, 32% P and 45% of S remained. The initial C:N, C:P and N:P molar ratios of the willow leaf litter were 24, 476 and 20, respectively. The factors governing the litter associated microbial activities were the chemical characteristics of the water and the quality of the leaf litter. The fungal biomass demonstrated a significant negative correlation with the remaining AFDM, the C:N ratio of the leaf litter and with the pH of the water, which suggested that the contribution of fungi during the breakdown of willow leaf litter was significant and the limiting nutrient was the nitrogen.

Keywords: Decomposition, leaf litter quality, willow, floodplain, oxbow lake, microbial activity

Introduction

In large rivers floodplain ecosystems, where the hydrological regime is the main driving force of the exchange of organic matter between the river and its adjacent floodplain, leaf litter has a major role in the lateral input of energy and nutrients from terrestrial to aquatic habitats and it represents an important part of the allochthonous organic matter (Junk *et al*. 1989, Chauvet 1997). Decomposition of leaf litter is an important ecosystem process and involves two main phases: the leaching of soluble compounds and the microbial mineralisation of structural components, achieved via microbial enzymatic activities and with an important role in the input of the terrestrial carbon into the aquatic detritus-based food webs (Langhans *et al*. 2008). Leaf litter decomposition is controlled by several biotic and abiotic factors, such as litter quality, substrate availability (C:N, C:P, N:P ratios), decomposer community, physical and chemical parameters of the water and hydrological regime (Gessner, Chauvet 1994). The composition and structure of riparian vegetation influences the litterfall and the particulate input. The breakdown of the leaf litter of willow *Salix alba* (L), a tree species that usually occurs immediately adjacent to the water bodies, has received an appreciable attention in the decomposition studies (Chauvet, Décamps 1989, Chauvet 1987, Chauvet 1997).

The aim of the study was to determine: (1) the decay rate and the N and P mineralisation rate of the willow leaf litter, (2) changes in leaf litter quality and microbial activity during the decomposition and (3) the effect of leaf litter quality and the environmental factors on the leaf litter associated microbial activity.

Study Site

The study site was located in Nyéki-Holt-Duna/ Nyéki Danube Oxbow Lake (NYHD) (N46°11.489' E18°50.937'), which is a backwater of the Danube River at Gemenc forested floodplain with an area of a 180 km^2. It is situated between the rkm 1469 and 1498 of the Danube River in the Danube – Drava National Park, Hungary (Fig. 1A). The threshold level of the surface hydrological connection of NYHD up- and downstream is reached when the water level of the Danube River at rkm 1478.7 (Baja gauge station) is 520 cm and 570 cm (Fig. 1B), respectively. Upstream it is achieved through Vén – Duna (VDU), Cserta – Duna (CSDU) and Sárkány – fok (SÁF), while downstream – through the Felső – Címerfok (CIF) (Schöll *et al*. 2009). Its channel is silting up and it has a high macrophyte cover.

Material and Methods

Leaf decomposition was measured by using the litter bag method. The willow (*Salix alba*) leaves were collected on 14 October, 2008, before their natural abscission by gently shaking the trees. The leaf litter was dried at room temperature and stored until the exposition. Three different sets of litter-bags with 25x20 cm size and 1 mm mesh size were prepared. Each litter bag contained 8 g of willow leaf litter and was placed out in NYHD on 09 June, 2009. Three bags were retrieved at each sampling date from 09 June, 2009 till 27 October, 2009 (Fig. 1). After the invertebrates were removed and preserved, the leaf material was carefully washed with tap water to remove any accumulated silt and to prevent loss of leaf material.

Litter chemical and microbial analyses

Subsamples of the remaining leaf material were dried at 105ºC to determine the dry mass (DM), then they were combusted at 550ºC, for four hours for determination of ash content. After that, the ash free dry mass (AFDM), which is as an index of the organic matter content, was calculated. The dried leaf material was ground with a motor mill for the determination of nutrient content. The C, N and S concentrations were evaluated by using a NCS analyser (NA-1500, Fisons Instruments). The P concentration was determined by spectrophotometric molybdenum blue method, after digestion with concentrated sulphuric acid. The C, N, P and S contents were calculated by multiplying each of the concentrations (mg g^{-1}) by the remaining mass (g). The remaining dry mass, ash free dry mass and the remaining amount of C, N, P and S at each sampling date were expressed as a percentage of the initial amount. The microbial parameters were determined from fresh subsamples. The electron transport assay, based on the tetrazolium reduction test, was used to determine the potential microbial respiratory activity (Packard 1971, Szabó 2003). The litter associated fungal biomass was estimated by extracting and quantifying ergosterol from the subsamples (Gessner, Newel 1997).

Mathematical and statistical evaluation of the data

The single exponential model: $X_t = X_0 \exp(-kt)$ was fitted to the data (Olson 1963), where X_t is the amount of AFDM, C, N or P, at time t; X_0 is the initial amount of AFDM, C, N or P; and k is the exponential breakdown (k_{DM}, k_{AFDM}) or nutrient release rate (k_C, k_N, k_P, k_S). For function fitting was used the Nonlinear Estimation method , which uses the approaching method of Levenberg-Marquardt. The Pearson product moment correlation analysis of the data was performed. The significance of the differences between the decay and release rates was assessed by T-test for Independent Samples for Variables. Significance level was evaluated at 0.05 in all cases. Statistical analyses were carried out with Statistica 6 programme package (StatSoft, Inc. 2001).

Physical and chemical analyses of the water

Temperatures, electrical conductivity and pH of the water were measured *in situ* with WTW field equipment. The total organic carbon (TOC), total carbon (TC) and total nitrogen (TN) concentrations were determined in the laboratory by TOC analyser (Elementar-liqui TOC). The NO_3-N concentrations were determined with DX-120 ionchromatograph (Dionex), while the PO_4-P, total phosphorus (TP), NH_4-N, suspended matter (SPM) and chlorophyll-a (Chl-a) concentrations were determined using standard chemical methods (Golterman *et al.* 1978). The water level data of the Danube River at rkm 1479 were obtained from http://www.hydroinfo.hu/. The Fluvial Connectivity Quotient (FCQ) was calculated for the investigation period: FCQ = number of flooded days/number of isolated days according to Poi de Neiff *et al.* (2006).

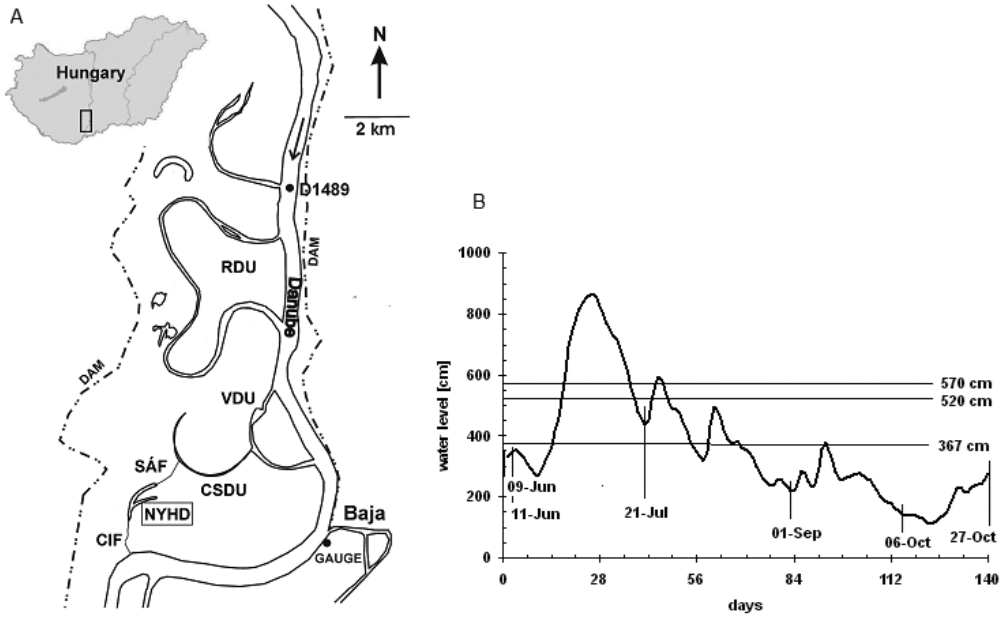

Fig. 1. Location of the study site Nyéki – Holt – Duna/ Nyéki – Danube Oxbow Lake (NYHD) at Gemenc floodplain (A) and the changes in water level of the Danube River (rkm 1479) at the gauge of Baja during the investigation period (mean water level: 367 cm, surface hydrological connectivity threshold levels of Nyéki-Holt-Duna at upstream (520 cm) and at downstream (570 cm)) and the sampling dates (B)

Results and Discussion

Environmental background conditions

The water of NYHD had lentic character during the majority of the days of the investigation period and the intensity of the connectivity, based on the fluvial connectivity quotient: FCQ = 0.23 was low (Fig. 1B). The minimum and maximum values of the main physical and chemical parameters of the water are presented in Table 1.

Mass loss

At the beginning, in the first 48h, the willow leaf litter had lost 19% from its initial AFDM and only 31% of the initial litter remained by the end of the decomposition. Webster *et al.* (1999) found that the chemical leaching of dissolved material accounts for 10-30% of the initial weight loss from most leaf species. The loss of mass followed a simple negative exponential pattern during the 140 day of the decomposition period. There were no significant differences ($p>0.05$) between the exponential breakdown rates of DM (k_C=0.0098±0.0007 d^{-1} and k_{AFDM}=0.0110±0.0009 d^{-1}) (Fig. 2, Table 2). The k_{AFDM} was comparable with the values reported by Baldy *et al.* (1995), k = 0.0091 d^{-1}, but it was approximately two times higher than that obtained by Chauvet (1987), k = 0.0054 d^{-1} for willow leaf litter decomposing in mesh bags with 2 mm mesh size in the Garrone River. These differences might be, on the one hand, owing to the differences in the environmental conditions. On the other hand, the sediments that were trapped in the bags or attached to the leaves, which were not removed, therefore may reduce the development of microorganisms, in this way may slow down the decomposition (Chauvet 1987).

Nutrient dynamics in decomposing leaf litter

The amount of C, N, P and S decreased during the investigation period, except for the last sampling date (140th day, 27 October, 2009) in the case of C, N and P, and the 84th day in the case of S, when an

Table 1. Changes in the main physical and chemical parameters of the water during the decomposition period

	T	pH	Cond.	NH_4-N	NO_3-N	PO_4-P	TOC	TC	TN	TP	SPM	Chl-a
	C°		µScm^{-1}	µgl^{-1}	mgl^{-1}	µgl^{-1}	mgl^{-1}	mgl^{-1}	mgl^{-1}	µgl^{-1}	mgl^{-1}	µgl^{-1}
min	16.0	7.3	339	1.1	0.1	11.7	16.0	59.2	2.0	131.1	1.5	11.5
max	27.0	8.4	482	109.2	0.3	129.9	23.2	79.2	3.0	306.5	29.5	90.6
mean	22.5	7.7	431	35.9	0.2	53.3	18.6	70.5	2.4	204.2	11.1	43.8

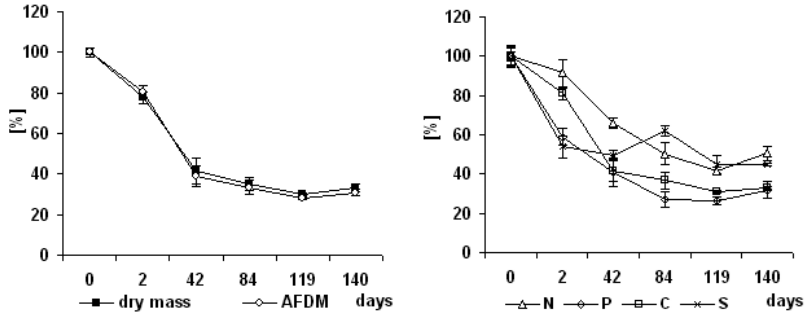

Fig. 2. Changes in the remaining dry mass, ash free dry mass (AFDM) and the remaining amount of C, N, P and S during the decomposition of *Salix alba* (L) leaf litter (mean±1SE, n = 3)

immobilisation was observed (Fig. 2). The increase in the remaining amount of N, P and C by the end of the decomposition can be attributed to the uptake of nutrients by the litter associated microorganisms from the surrounding water. It may be owing to their microbial demand for these elements and the incorporation of nutrients in their own biomass. The nutrient dynamics associated with leaf litter depends on the activity of microorganisms, which increased by the end of the decomposition (Fig. 3). Chauvet (1987) found a decrease in the amount of P below the initial levels during the decomposition, which he explained with the gradual disappearance of the leaf material, and with an increase in the amount of N and organic P on the 28th and 119th day of decomposition, as compared to the initial values, which was attributed to the import of N by the litter associated microorganisms and by the deposition of the sediment on the leaf material, respectively.

The decomposition pattern and the k-values obtained for the C and AFDM were comparable and did not differ significantly (k_C = -0.0098±0.0007 d^{-1} and k_{AFDM} = -0.0110±0.0009 d^{-1}, Table 2). Our findings were comparable to the results of Chauvet (1987) who found that k_{AFDM} = 0.0054 d^{-1} and k_C = 0.00453 d^{-1}. In addition, he also found that the amounts of C changed in a similar pattern to that of the organic matter, while we obtained higher values.

The P decomposed with the fastest rate, followed by the C, then by the N, while the S decomposed with the slowest rate. The range of the nutrient release rates was: $k_P > k_C > k_N > k_S$ (Table 2). We found that 33% of C, 50% of N, 32% of P and 45% of S remained by the end of the decomposition (Fig. 2).

Table 2. Exponential breakdown and nutrient release rates: k (d^{-1}) of decomposing willow leaf litter in Nyéki – Holt – Danube (NYHD) (mean±1SE, n = 3).

	W_0			k			R^2		
AFDM	87.8	±	1.1	0.0110	±	0.0009	0.97	±	*0.01*
DM	86.2	±	1.3	0.0100	±	0.0009	0.97	±	*0.01*
N	93.8	±	4.2	0.0062	±	0.0006	0.99	±	*0.01*
P	77.7	±	1.1	0.0102	±	0.0018	0.93	±	*0.03*
C	87.5	±	1.3	0.0098	±	0.0007	0.97	±	*0.01*
S	74.6	±	1.5	0.0040	±	0.0004	0.94	±	0.02

Leaf litter quality

The initial C:N molar ratio of the willow leaf litter was 24±0.91. After the 48h leaching it decreased to 21, then it varied between 15±0.77 and 18±0.59 until the end of the decomposition period. The initial C:P and N:P molar ratios were 476±17.42 and 20±1.05, respectively. The C:P and N:P ratios exceeded the initial values at each of the subsequent sampling times, and they varied from 490-645 and 31-37, respectively (Fig. 3). Chauvet (1987) observed an initial C:N value of 25.4, which was similar with the value obtained by us. The C:N ratio rapidly decreased during the first 42 days then very slowly increased (Fig. 3). Chauvet (1987) observed a rapid decrease in the C:N ratio during the first month, then a very slow decrease until the 6th month; and a constant N:P ratio during the decomposition, which was also different from our findings (Fig. 3). The initial C:S ratio was 569±29.66 and exceeded the critical C:S ratio of 200-400 at which mineralisation takes place normally (Howlader *et al*. 2004).

Microbial activity

The ETS-activity ranged from 0.7 to 2.0 µl O_2 mg^{-1} h^{-1}. The decrease in ETS-activity in the first 48h may be explained by the decay of microorganisms with terrestrial origin. In summer months, especially in June and July when the water temperature raised, the ETS-activity showed the highest peaks (Fig. 3A).

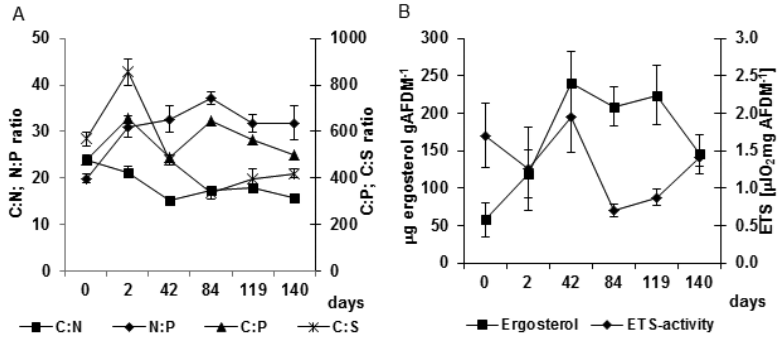

Fig. 3. Changes in C:N, N:P, C:P and C:S molar ratios (A) and ETS-activity and fungal biomass as ergosterol (B) during the decomposition period (mean±1SE, n = 3)

The changes in ETS-activity varied almost in parallel with the temperature of the surrounding water. Their correlation was r = 0.40, but it was not significant ($p > 0.05$). It is known that the activity of the microorganisms, which are involved in decomposition, is temperature dependent (Ágoston-Szabó *et al.* 2006). The reason for it might be that the microbial activity could be affected by the substrate availability interacting with the temperature. The availability of organic substrates might cause the running down of microbial activity by the end of the decomposition. A negative correlation (r = -0.88, p = 0.02) was found between the ETS-activity and the TC concentration of the water.

The ergosterol concentration ranged from 57.5 to 239.6 µg g^{-1}. The fungal biomass sharply increased and it showed the highest peak on 42th day of the decomposition (on 21 July). The period of the highest ergosterol concentration has been found to occur between day 42 and day 119 (Fig. 3B). Baldy *et al.* (1995) measured peak ergosterol concentrations (0.50 mg g^{-1} AFDM) earlier than us, after the fourth week of the decomposition.

The ergosterol concentration showed a significant negative correlation with the remaining AFDM (r = -0.86, p = 0.03), C:N ratio (r = -0.83, p = 0.04) of the decomposing leaf litter and with the pH of the water (r = -0.87, p = 0.02), which suggested that the contribution of fungi in the breakdown of the willow leaf litter was significant and their limiting nutrient was the N.

Conclusions

Our results, which represent a part of the dataset from the decomposition studies conducted at Gemenc floodplain, can have implications for the nutrient management of this ecosystem. We concluded that the leaf litter of *Salix alba* (L.) is a valuable carbon and nutrient source for the litter associated microbial communities, which play an important role in decomposition of leaf litter in standing water bodies of fluvial systems, by governing the microbially mediated energy flow from leaf litter to higher trophic levels. The main regulating factors governing the decomposition were the litter quality, the pH and temperature of the surrounding water.

Acknowledgements: This work was supported by the Deutsche Bundesstiftung Umwelt, AZ 24050 project and by the Hungarian Academy of Science. We thank Buzetzky Győző for his help in the fieldwork, and Gábor Horváth and Bernadett Garad for the chemical analyses. The authors are grateful to Hristina Kalcheva and Svetlana Naumova reviewing this manuscript.

References

Ágoston-Szabó E., M. Dinka, L. Némedi and G. Horváth 2006. Decomposition of *Phragmites australis* rhizome in a shallow lake. – *Aquatic Botany*, **85**: 309-316.

Baldy V., M. O. Gessner and E. Chauvet 1995. Bacteria, fungi and the breakdown of leaf litter in a large river. – *Oikos*, **74**: 93-102.

Chauvet E. 1987. Changes in chemical composition of alder, poplar and willow leaves during decomposition in a river. – *Hydrobiologia,* **148**: 35-44.

Chauvet E. 1997. The leaf litter decomposition in large rivers: The case of the River Garronne. – *Limnetica*, **13** (2): 65-70.

Chauvet E., H. Décamps 1989. Lateral interactions in a fluvial landscape: the River Garonne, France. – *Journal of North American Benthological Society*, **8**: 9-17.

Gessner M. O., E. Chauvet 1994. Importance of stream microfungi in controlling breakdown rates of leaf litter. – *Ecology*, **75**: 1807-1817.

Gessner M. O., S. Y. Newell 1997. Bulk quantitative methods

for the examination of eukaryotic organoosmotrophs in plant litter. In: Hurst C. J., G. Knudsen, M. McInerney, L. D. Stetzenbach and M. Walter (eds.), Manual of Environmental Microbiology, ASM Press, Washington, D. C., USA, 295-308 pp.

Golterman H. L., R. S. Clymo and M. A. M. Ohnstad 1978. Method for physical and chemical analysis of freshwaters. IBP Handbook No. 8, Blackwell Sci. Publ., 213 pp.

Howlader M. A. R., A. R. N. Solaiman and M. A. H. Chowdhury 2008. Biodynamics of microbial biomass, nitrogen and sulfur in organic matter amended soil. – *Bulletin of the Institute of Tropical Agriculture Kyushu Univ*ersity, **31**: 19-29.

Junk, W. J., P. B. Bayley and R. E. Sparks 1989. The flood pulse concept in river floodplain systems pp. 110-127. in D. P. Dodge ed. Proceedings of the International Large River Symposium. – *Canadian Special Publication of Fisheries and Aquatic. Sciences*, **106**: 110-127.

Langhans S. M., S. D. Tiegs, M. O. Gessner and K. Tockner 2008. Leaf-decomposition heterogeneity across a riverine floodplain mosaic. – *Aquatic Sciences*, **70**: 337-346.

Olson J. S. 1963. Energy storage and the balance of producers and decomposition in ecological systems. – *Ecology*, **44**: 322-330.

Packard T. T., M. L. Healy and F. A. Richards 1971. A vertical distribution of the activity of the respiratory electron transport system in marine plankton. – *Limnology and Oceanography*, **16** (1): 60-70.

Poi de Neiff A., J. J. Neiff and S. L. Casco 2006. Leaf litter decomposition in three wetland types of the Paraná River floodplain. – *Wetlands*, **26** (2): 558-566.

Schöll K., A. Kiss, M. Dinka , E. Ágoston-Szabó, Á. Berczik, A. Schmidt and G. Fehér 2009. A Gemenci hullámtér víztereinek hidrobiológiai különbségei (Duna-Dráva Nemzeti Park – Hydrobiological differences between the water bodies of the active floodplain of Gemenc. (In Hungarian); http://apps.arcanum.hu/hidrologia/a111126.htm?v=pdf&q=(SZO%3D(sch%C3%B6ll))%2FHIDRVAND&s=SORT&m=0&a=rec#pg=328&zoom=f&l=s

StatSoft, Inc. 2001. STATISTICA (Data Analysis Software System), Version 6; www.statsoft.com.

Szabó E. 2003. The use of the tetrazolium reduction test for the detection of the terminal electron transport system (ETS) activity in decomposing reed (*Phragmites australis* /Cav./ Trin. ex Steud.) rhizome. – *Annales de Limnologie – International Journal of Limnologie*, **39** (1): 63-70.

Webster J. R., E. F. Benfield, T. P. Ehrman, M. A. Schaeffer, J. L. Tank., J. J. Hutchens and D. J. D. Angelo 1999. What happens to allochthonous material that falls into streams? A synthesis of a new and published information from Coweta. – *Freshwater Biology*, **41**: 687-705.