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RESEARCH ARTICLE

Decomposition of reed swamp detritus in the Danube Delta: a case study of four eutrophic systems

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Abstract

1 - Leaf litter decomposition rates in aquatic ecosystems are known to be related to many abiotic and biotic factors.

2 - Here, field experiments were carried out during fall 2005 in four sites of the Danube Delta system, differing mainly in terms of type and characterised by euthrophycation.

3 - The litter bag technique was used to study reed decomposition and evaluate the relevance of ecosystem characteristics to reed leaf decay rates.

4 - Trophic conditions were very high in all sites (TRIX > 8.0), indicating poor water quality. Overall, the processing rate k of *Phragmites australis* in the Danube Delta was equal to 0.016 days^{-1}.

5 - The mass remaining in the leaf bags differed among ecosystems (ANOVA, $P < 0.001$) with higher decay rates in Isac lake ($k = 0.024$) than in the other systems. P. australis leaf processing varied considerably in the studied lakes and channel (cv = 30.7%).

6 - A high percentage (>60%) of leaf breakdown variability was related to differences in phosphorus compound concentrations in the water.

Keywords: Danube Delta, *P. australis*, decomposition, eutrophication.

Introduction

Submerged and littoral macrophytes, especially reed stands, are important contributors to primary production in freshwater and transitional aquatic ecosystems (Mann, 1972). In these ecosystems, only a small part of aquatic macrophyte production is directly consumed by herbivores (Mann, 1975), a large part of the macrophyte biomass having a major function in the detritic pathway (Cummins *et al*. 1973, Webster and Benfield 1986). These inputs are made available through decomposition, a key ecosystem process that enables the recycling of nutrients and chemical elements and sustains important food chains composed of organisms that use this resource (Cebrian 1999, Takeda and Abe 2001).

Microorganisms and macroinvertebrates are the agents of decomposition of dead organic matter (Rossi, 1985; Gessner and Chauvet, 1994), reducing it to elements that can be released into the system (Valiela *et al*. 1985, Gessner *et al*. 1999); moreover, many physical and chemical factors have been found to affect plant decomposition rates. The factors include characteristics of the plant species and the leaves (Kok *et al*. 1990, Canhoto and Graça 1996), ecosystem characteristics such as water temperature and salinity (Carpenter and Adams 1979, Reice and Herbst 1982, Vought *et al*. 1998), pH (Thompson and Bärlocher 1989), nutrient levels (Elwood *et al*. 1981, Sharma and Gopal 1982), and regional characteristics such as climate (Murphy *et al*. 1998) and solar radiation (Denward and Tranvik 1998).

The decomposition of organic matter has been cited as a major source of energy for transitional aquatic ecosystems (Mann 1972, Valiela 1984), which are ecotones, functionally connecting the land and its rivers on one side to the sea on the other (Wiegert and Pomeroy 1981).

Transitional aquatic ecosystems couple continental to marine environments, receiving active bio-geochemical inputs from the land, rivers and coastal seas. They are often extremely productive systems, supporting high rates of primary production and consequently large quantities of organic matter to be decomposed.

Many studies have examined the relationships between water chemistry and plant organic matter decomposition in aquatic environments such as streams (Menendez *et al*. 2001; Gulis *et al*. 2006) and lakes (Carpenter and Adams 1979). There is evidence that decomposition can be regulated by dissolved nutrients, as shown by leaf decomposition studies in eutrophic lakes (Royer and Minshall 1997), rivers differing in nutrient concentrations (Meyer and Johnson 1983) or in reference and eutrophic streams (Gulis *et al*. 2006); moreover, the results also vary in controlled experimental studies (Bayley *et al*. 1985, Robinson and Gessner 2000, Royer and Minshall 2001), and the effects of dissolved nutrients on detrital food chains, i.e., on rates of plant detritus breakdown, are not clearly understood (Suberkropp and Chauvet 1995).

In this context, the Danube Delta is a good example of a transitional aquatic ecosystem in which to investigate the relationships between the chemical characteristics of the water and plant litter decomposition. Several authors (Vadineanu and Cristofor 1987, Cristofor *et al*. 1993) have reported increasing nitrogen and phosphorus loads in the Danube River over the last few decades, with increasing eutrophication of some areas, leading to significant changes in ecosystem characteristics.

The Danube Delta is one of the most important wetland areas in Europe because of its ecological value, surface area (5800 Km^2) and geographical position at the terminal zone of the second largest river in Europe (Rîşnoveanu *et al*. 2004); moreover, it is one of the most extensive reed bed systems in Europe. The 5800 Km^2 Danube Delta system (1130 Km^2 permanently covered by water) receives waters from the Danube River and the Black Sea in flooded wetlands. The Danube Delta has decreased by 25% since 1965; consequently, the filtering potential of this system has also decreased and its nutrient content has increased. In fact, most superficial waters in Romania are classified as mesotrophic and eutrophic environments by the Report on the State of the Environment in Romania (Ministry of Environment and Water Management, 2003), in the moderate and poor quality classes on the basis of Law n° 310/2004, annex 11.5 (Schmüdderich and Platon 2007).

For this study we chose four systems differing in type (three lakes and one channel) and water chemical characteristics. The decomposition processes were studied by analyzing detritus mass loss in the ecosystems and by evaluating the relevance of water chemical characteristics to reed leaf decomposition rates. The main aims of the study were to acquire information on the influence of water chemistry on the rate of plant detritus processing in aquatic ecosystems and to give an estimate of plant detritus decomposition in the Danube Delta, as a key ecosystem process useful to evaluate the health of this valuable ecosystem.

Material and methods

Study sites

The study took place in four transitional aquatic ecosystems lying within the Danube Delta in South-Eastern Romania. Before entering the Black Sea, the Danube forms a wide branching delta, located between 28°45' E and 29°46' E longitude and 44°25' N and 45°37' N latitude; a mosaic of shallow lakes and channels, fringed by reed beds, lies between the delta's initial three branches. Four of these ecosystems, located in the Gorgova-Uzlina complex, were included in this study: (from North to South) Cuibul cu Lebede lake (45˚08'N, 29˚20'E), the Isac II channel (45˚07'N, 29˚17'E), Isac lake (45˚06'N, 29˚16'E) and Uzlina lake (45˚05'N, 29˚15'E).

Cuibul cu Lebede is classified as a 'medium' type lake with an area of 1-10 km^2; it is a eutrophic hard-water lake located close to the navigable Litcov channel, surrounded by reed beds with abundant aquatic vegetation. Isac II is a small channel which connects Isac lake with the Litcov channel. Isac lake, situated in the lower-lying eastern part of the Gorgova-Uzlina complex, is a 'large' type lake with an area of 10-100 km^2. Uzlina lake, situated very close to the southern Danube branch (Sfantul Gheorghe), is a 'medium' type lake with an area of 1-10 km^2; it is particularly influenced by Danube inputs from the Sfantul Gheorghe branch.

Leaf-bag experiments

The study of organic matter decomposition in

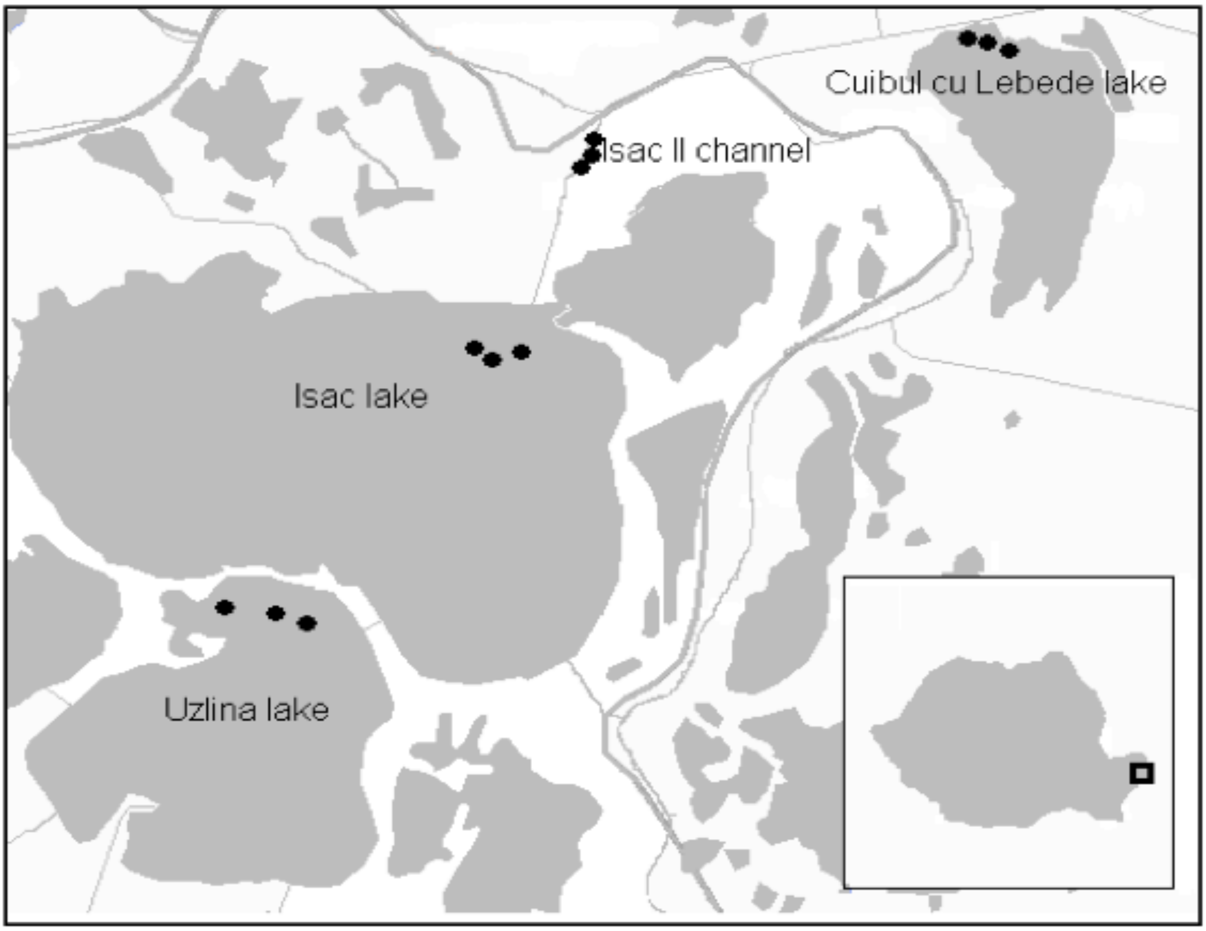

Figure 1. Study sites in Danube Delta system with location of sampling stations for each ecosystem.

the Danube Delta was realised using leaves of Phragmites australis (Cav.) Trin. ex Steud. The research was carried out during fall in four ecosystems, each with three sampling stations and three replicates per station.

The leaf decomposition process was studied using the leaf bag technique (Bocock and Gilbert 1957, Petersen and Cummins 1974). Leaves were collected simultaneously and from the same area at the beginning of fall; the basal and apical parts of the leaves were cut off and only the central leaf section was used. An estimate of the leaves' initial ash free dry weight was made from sub-samples. In late fall, litter bags (0.5 cm mesh size) were filled with 3.000±0.005 g of oven-dried leaves (60°C, 72 h); nine leaf bags were placed at each sampling station and collected after 15, 30 and 45 days from the beginning of the experiment.

At each sampling time, three leaf bags were sampled from each sampling station, placed in a separate plastic container, and rapidly taken to the laboratory, where the leaves were gently washed to remove sediments. Leaves from each bag were oven-dried at 60 °C for 72 h, weighed and combusted (500 ° C, 6 h) to obtain the ash content.

For each ecosystem, information on physiographic characteristics (surface area and depth) was obtained. Data on physicochemical water parameters were collected during a monitoring program conducted four times a year from 2000 to 2005. These include data on water transparency, dissolved oxygen, pH, temperature, nitrogen compounds, phosphorus compounds and chlorophyll-*a*. The water's physical characteristics were monitored using a multi-probe (YSI 556 MPS), while nutrient and chlorophyll-*a* concentrations were determined in accordance with Strickland and Parsons (1972) and Yentsch and Menzel (1963) respectively.

Moreover, the Trophic Index (TRIX) was also calculated as a linear combination of the logarithms of four variables: chlorophyll-*a*, oxygen saturation, dissolved inorganic nitrogen and dissolved total phosphorus (Vollenweider *et al*. 1998).

Data analysis

Data were tested for conformity to assumptions of variance homogeneity (Cochran's test) and arcsine-transformed where necessary.

On Phragmites australis leaves, the percentage of the original mass remaining at the time of sampling was estimated (Petersen and Cummins, 1974). Processing rates were then evaluated by assuming an exponential model of mass loss utilizing M_0 (initial mass), M_t (mass at sampling time) and t (time) values to estimate k (days^{-1}) (Olson 1963).

Three-way nested analysis of variance (ANOVA) was used to test for differences in leaf decay rates in space (i.e., among sites and sampling stations) and time (i.e., among sampling times). The slopes of regression equations for the mass-loss data from each ecosystem were compared by analysis of covariance (ANCOVA).

The relevance of chemical characteristics as potential sources of variation in leaf decay rates was analyzed using multiple regression analysis.

Results and discussions

Table 1 shows the main physiographic characteristics of the ecosystems and water physicochemical parameters monitored from 2000 to 2005. The water bodies varied widely in terms of surface area, ranging from 0.01 km^2 for the Isac II channel to 10.20 km^2 for Isac lake. The physicochemical parameters varied less sharply among water bodies; dissolved inorganic nitrogen as ammonium and nitrates was similar in all ecosystems, while nitrites ranged between 0.046 mg/l in Isac lake and 0.205 mg/l in Uzlina lake; phosphorus as phosphates ranged between 0.032 mg/l in Isac lake and the Isac II channel

Table 1 - Structural ecosystem characteristics and physicochemical water parameters of each studied site in Danube Delta system. Ecosystems are listed from most northerly (Cuibul cu Lubede) to most southerly (Uzlina).

Parameters	Unit	Cuibul cu Lebede l.	Isac II channel	Isac lake	Uzlina lake
Surface	km^2	1.96	0.01	10.20	4.83
Depth	cm	155	200	209	159
Transparency	cm	111		83	59
Dissolved oxygen	mg l^{-1}	11.1	9.07	13.0	11.5
pH		8.4	8.0	8.5	8.5
Temperature	°C	18.8	18.4	19.9	19.0
$N(NH_4)$	mg l^{-1}	0.554	0.659	0.479	0.460
$N(NO_2)$	mg l^{-1}	0.107	0.189	0.046	0.205
$N(NO_3)$	mg l^{-1}	0.476	0.531	0.546	0.568
TP	mg l^{-1}	0.134	0.154	0.138	0.152
$P(PO_4)$	mg l^{-1}	0.037	0.033	0.032	0.051
Chl *a*	mg m^{-3}	38.32		51.60	55.73
TRIX		8.60		8.72	8.82

and 0.051 mg/l in Uzlina lake. Considerable variation among ecosystems was observed for chlorophyll-a concentrations, which ranged between 38.32 mg/m^{-3} in Cuibul cu Lubede and 55.73 mg/m^{-3} in Uzlina lake. Moreover, the trophic conditions of the Danube Delta system were found to be very high for all ecosystems, with the Trophic Index (TRIX) more than 8.0 in all cases, indicating bad water quality with high eutrophication (Table 1).

The temporal variability of nutrients, as measured in the 2000-2005 monitoring program and expressed as coefficient of variation (%), was observed to be higher than that of physicochemical parameters in all sites; the coefficient of variation was 104.8 and 30.5% % for nutrients and physicochemical parameters respectively (Table 2).

Table 2 - Temporal variability (from 2000 to 2005), expressed as coefficient of variation (%), calculated on physicochemical data (transparency, temperature, dissolved oxygen, pH,) and nutrients (ammonia, nitrates, nitrites, inorganic phosphorus, phosphates and chlorophyll-a) for each studied site.

	Coefficient of variation (%)	
	Physicochemical parameters	Nutrients
Cuibul cu Lebede l.	30.7	107.9
Isac II channel	32.9	106.5
Isac lake	30.7	108.4
Uzlina lake	27.7	96.6

According to Vollenweider (1982) lakes are eutrophic when the following conditions apply: chlorophyll-a concentration > 7 µg l^{-1}, TDP > 1.6 µM and nitrogen > 64 µM. In our sampling, all three investigated lakes exceeded the limits for both chlorophyll-a and total dissolved phosphorus concentrations. Specifically, chlorophyll-*a* content can be used as a descriptor of the trophic state and productivity of most estuarine systems (Cahoon and Cooke 1992, de Jong and de Jonge 1995). In this case, chlorophyll-*a* concentrations were very high, and comparable to those reported in highly productive coastal systems such the North Sea (Boon *et al*. 1998) and in coastal lagoons (Conde *et al*. 1999, Garrigue 1998). Since all these environments have been classified as eutrophic, this simple observation suggests that the studied area of the Danube Delta system is highly eutrophic, which could be verified with reference to other trophic state indicators such as phytoplankton biomass.

This becomes even more evident when we consider the tropic index (TRIX), which is very high for all ecosystems. Similar results were obtained in previous studies (Friedrich *et al*. 2003), confirming increased nutrient loads in the Danube Delta system over the last few decades (Cociasu *et al*., 1996) compared to previous results (Almazov 1961). Overall, P. australis decomposition in the Danube Delta system fits a negative exponential model ($y = 81.96\ e^{-0.016x}$; r = 0.83; df = 10; P<0.001). Considering all sampling stations in the four ecosystems, we calculated an average daily reed detritus mass loss of 1.57% and a reed detritus half-life of 44 days.

P. australis has received considerable attention because it is an important primary producer in aquatic ecosystems (Denward and Tranvik 1998, Rossi and Costantini 2000, Gessner 2000, Sangiorgio *et al*. 2006). Until this study however, *P. australis* decomposition rates in the Danube Delta had not been investigated. Reed leaf decomposition processes have been investigated in different ecosystem types, with decay rates ranging from slow to fast, depending on various factors including climate. However, little or no research has been undertaken into litter decomposition processes in the Danube delta. In terms of Petersen and Cummins' classification (1974) *P. australis* in the Danube Delta was found to be "fast"; decay rates of reed leaves in each studied site were comparable with those observed in a previous study carried out in Black Sea transitional aquatic ecosystems (Sangiorgio *et al*. 2008). On the entire data set, considering sites, stations and sampling times as sources of heterogeneity, temporal variation of reed decomposition was found to be significantly more important than spatial (three-way ANOVA, P<0.001) (Table 3).

Moreover, the remaining mass of *P. australis* leaves varied significantly among ecosystems, with reed leaves decaying faster in Isac lake (k = 0.024) than in the other

Table 3 - Three-level nested ANOVA (stations nested within sites) testing for effects of sites, stations and sampling times on remaining mass (%) of *P. australis* leaves in the studied ecosystems. (*** P < 0.001).

Source	df	MS	F	P
Sites	3	0.1831	12.41	***
Field stations (sites)	8	0.0748	5.07	***
Days	2	1.7101	115.93	***
Error	90	0.0148		

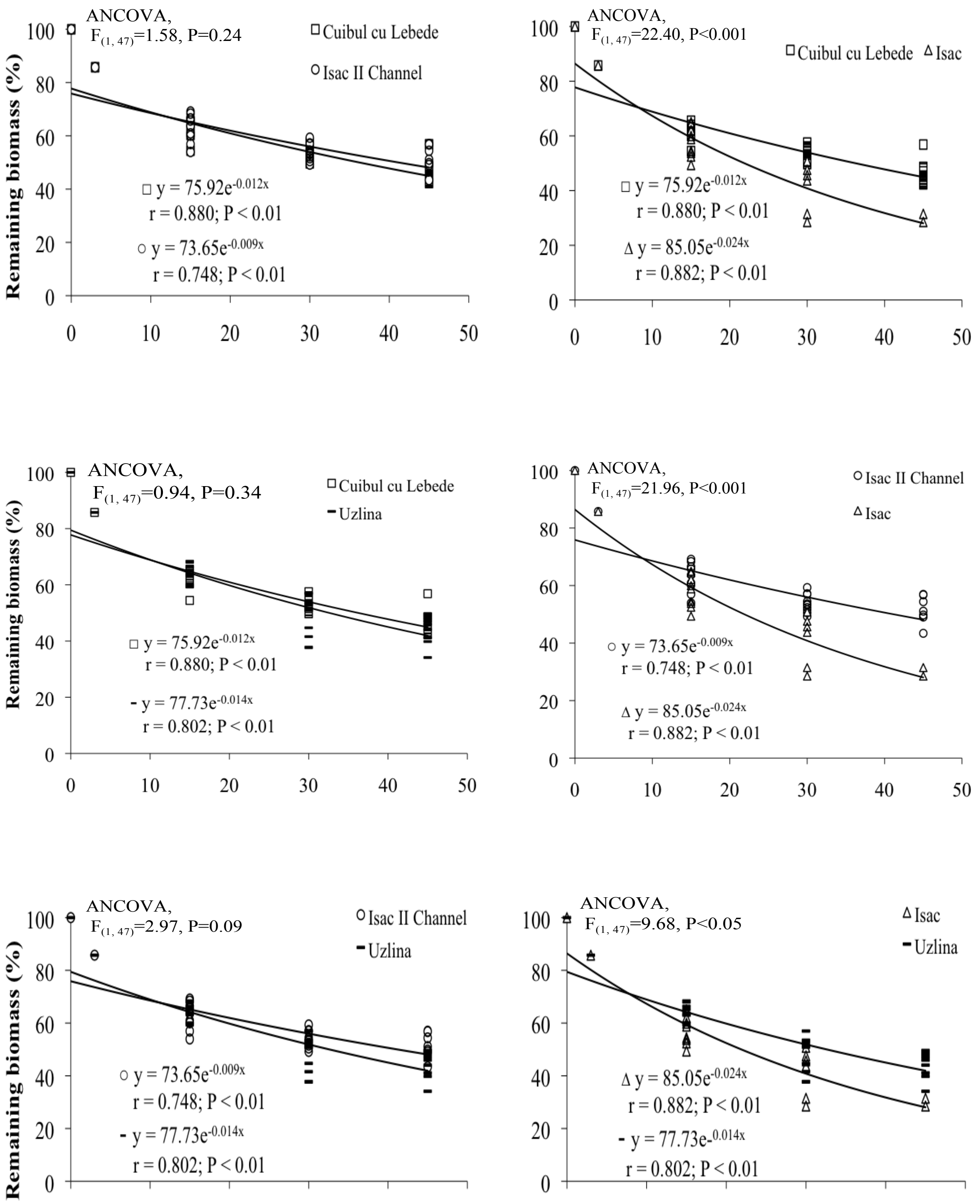

Figure 2. Statistical comparisons (ANCOVA) of temporal variation of *P. australis* leaf mass in all combinations of ecosystems from most northerly (Cuibul cu Lubede) to most southerly (Uzlina).

lakes (k [Cuibul cu Lebede] = 0.012 and k [Uzlina] = 0.014) or the Isac II channel (k = 0.009) (ANCOVA, Figure 2).

Comparative studies of reed decomposition processes in various types of ecosystem report faster leaf decay rates in lotic ecosystems, such as stream and rivers, than in lentic ones, such as lakes and lagoons (Cortes *et al*. 1995, Sangiorgio *et al*. 2004). The spatial pattern of reed decomposition observed in the present study may be related to the surface area of the ecosystems. The pattern of leaf breakdown rates in the Danube Delta system (i.e., the Isac channel < Cuibul cu Lubede < Uzlina < Isac lake) reflects the relative surface area of the studied ecosystems. The influence of 'island area' on ecosystem properties and consequently on the functioning of ecosystems is well known (Wardle *et al*. 1997).

There is evidence that increasing taxonomic richness and individual abundance in aquatic ecosystems are related to increased leaf decay rates (Robinson *et al*. 1998), and that larger ecosystems have more species (Connor *et al*. 1979). In this case, there is no information available on biotic agents of decomposition, such as macroinvertebrates, so the relationships between ecosystem surface area and ecosystem functioning can only be conjectured.

To obtain an estimate of the variability of *P. australis* decay rates at different spatial scales, the coefficient of variation from the ecosystem level to that of leaf bag replicates was calculated. Overall, considering all field stations and sampling times, the coefficient of variation based on decay rate data was 30.7%. As expected, a higher variability was observed at ecosystem level (cv = 21.0%), than among leaf bag replicates (cv = 8.5%); however, reed decomposition rates varied more considerably after 30 days of leaf bag submersion than at other sampling times (Table 4). Considering all sampling stations and times, *P. australis* leaf breakdown rates were found to vary significantly as a function of phosphorus compounds (stepwise multiple regression analysis, $r^2 = 0.68$, s.e. = 0.003, $F_{2,9} = 9.38$, $P < 0.01$), covarying with total phosphorous ($B = 0.53$, $P < 0.01$).

Table 4 - Spatial variability, expressed as coefficient of variation (%), calculated on decay rate data at different spatial scales (Cuibul cu Lebede lake, Isac II channel, Isac lake, Uzlina lake) for each sampling time.

	Coefficient of variation on decay rate data (%)			
Times	15	30	45	
Among sites	17.0	25.3	20.6	**21.0**
Among stations	14.8	16.1	11.4	**14.1**
Among replicates	6.2	8.4	10.7	**8.5**

Many studies have shown significant relationships between the chemical characteristics of the water and reed decomposition rates (Abelho and Graça 2006, Menéndez *et al*. 2001).

Several authors observed increased decay rates in conditions of nutrient enrichment (Elwood *et al*. 1981, Moss and Suberkropp 2001, Bärlocher and Corkum 2003), while in other cases no differences were found between fertilized and unfertilized leaf packs (Robinson and Gessner 2000, Royer and Minshall 2001).

In the Danube Delta, in terms of abiotic factors, the high trophic conditions and nutrient availability may play a key role in enhancing leaf decomposition rates. Even though the ecosystems differed only slightly in terms of chemical parameters, big differences in reed decomposition rates among the studied sites were observed. This also suggests that leaf litter decomposition is sensitive to even minor changes in eutrophication. In this regard, further evidence of the stimulation of leaf decomposition rates by even moderate eutrophication in aquatic environments can be found in the literature. The relationships often depend on the leaf species and on the tendency of higher nutrient levels to enhance biotic agents (i.e., microorganisms and invertebrates); in contrast, mesh size has been found to have little importance (Gulis *et al*. 2006).

Conclusions

In conclusion, the results of the present study are consistent with two main points:
(1) *P. australis* reed leaves in the Danube Delta system are characterized by fast decay rates; (2) the high decomposition rates of *P. australis* and the high variability related to water chemical parameters confirm the role of abiotic ecosystem characteristics as ecological forcing factors driving decomposition processes in the studied ecosystems.
Moreover, the present investigation emphasizes the usefulness of organic matter decomposition in studies of ecosystem functioning and ecosystem health.

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