



The litter bag technique for studying detritus decomposition in aquatic ecosystems. A case study in the South of Italy (Lake Alimini)

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Abstract Leaf litter decomposition rates in aquatic ecosystems are known to be related to many different abiotic and biotic factors. A comparative analysis of inter- and intra-habitat variations of detritus decay rates across ecosystem types was carried out in the Alimini lake complex (Italy) to assess the relevance of major ecosystem features to detritus processing rates. Overall, the spatial variability of leaf decomposition rates was more pronounced than temporal variability, decomposition rates in the stream being 3.6 and 5.2 times faster than in the freshwater lake and saltmarsh, respectively. Overall, environmental features were relevant factors affecting intra- and inter-habitat variation of reed decay rates.

Introduction Allochthonous organic matter constitutes the main source of energy in aquatic ecosystems (Fisher and Likens 1973), which is made available through decomposition processes. In aquatic ecosystems, only a small part of submerged aquatic macrophyte production is directly consumed by herbivores (Mann, 1975), while a large part of macrophyte biomass has a major function in the detritic pathway.

Decomposition processes of plant detritus in aquatic ecosystems have received increasing attention in the last three decades. Most of these studies have been based on three major approaches, focusing on the influence of detritus decomposition on water chemistry (Gupta *et al.*, 1996), chemical changes in leaf detritus during processing (Robertson, 1987; Bärlocher *et al.*, 1995) and energetic aspects of detritus processing (Petersen and Cummins, 1974). The third approach was developed mainly utilizing detritus breakdown rates as descriptors of detritus processing (Gessner, 1991).

Plant breakdown rates in aquatic ecosystems have been found to be affected by internal factors, i.e. leaf

species and chemical-physical characteristics of the leaves themselves (Kok *et al.*, 1990; Canhoto and Graça, 1996), and by external environmental factors such as water temperature and salinity (Reice and Herbst, 1982), pH (Thompson and Bärlocher, 1989), nutrients (Sharma and Gopal, 1982), or regional characteristics such as climate (Murphy *et al.*, 1998) and solar radiation (Denward and Tranvik, 1998). In addition, plant decomposition rates have been described relative to biotic factors, highlighting the role of micro-fungi and invertebrates (Rossi, 1985; Gessner and Chauvet, 1994).

It has been proposed that leaf degradation rates could be used as a tool to evaluate environmental quality, as leaf litter breakdown is a complex process in which biotic and abiotic factors are involved (Graça, 1993). A thorough understanding of this process is critical, not just to grasp the essence of ecosystem functioning but also to predict the consequences of global environmental changes at various scales (Graça *et al.*, 2005). Given the significance of the process, it is not surprising that ecologists have studied litter decomposition at least since Darwin. Methods have since been substantially broadened and refined, although some basic approaches such as the mesh-bag technique are still useful and widely employed.

Here we describe very briefly the main steps of an experimental study on decomposition of plant organic matter in aquatic ecosystems with a simple study case of decomposition processes of *Phragmites australis* leaf detritus in the lake complex of Alimini (Italy).

Material and methods

Detritus processing of *P. australis* leaves can be studied using the leaf bag technique (Bocock and Gilbert, 1957; Petersen and Cummins, 1974; Melillo *et al.*, 1983), based on the estimate of mass loss of plant material from litterbags.

Leaves of *P. australis* need to be collected at the beginning of autumn, air-dried and stored in a dark room at standard temperature and low humidity until needed. Before use, leaves are cut into roughly 8-cm-long fragments and oven-dried to constant weight (60±°C for 72 h). Lots of 3±0.005g dry weight are placed in 5 mm mesh bags.

The ash free dry weight (AFDW) of leaf packs is determined on a sub-sample of leaf packs to obtain an estimate of the biomass at the beginning of the experiment. Leaf packs are anchored to the bottom with bricks, steel or pegs, and collected at different sampling times (e.g., 1, 3, 30 and 90 days) in according to a pre-planned schedule (depending on leaf type).

At each sampling time, some leaf packs (e.g., 5) are sampled, placed in a plastic container separately and rapidly brought to the laboratory. Here, leaves are gently washed to remove sediments and macroinvertebrate colonizers. Leaves from each pack are dried in an oven at 60°C for 72 h, weighed, burned in a muffle furnace at 500°C for 6 h and weighed again.

It is important to measure the physical and chemical characteristics of the water (e.g., dissolved oxygen, pH, salinity, temperature, nitrate, nitrite, ammonium and phosphate) at each station during the sampling period. Physical characteristics can be measured with field instruments in situ, while nutrient concentrations can be determined in the laboratory from water samples. Moreover, it is advisable to measure some structural ecosystem characteristics such as the organic content of the bottom sediment at each sampling station.

Mass-loss data can be processed using non-linear regression analysis of the exponential model (Olson, 1963):

$$M_t = M_0 e^{-kt}$$

where M_t is the mass remaining at time t , M_0 the initial mass and k the breakdown coefficient (days⁻¹); k values can be used as a measure of reed processing

rates. Moreover, it is possible to run an analysis of covariance using time as a covariate if data from more than one series have been collected.

Case study **Study area**



Figure 1 - Map of Lake Alimini

The study was carried out in the drainage basin of the lake complex of Alimini, in southern Italy on the Adriatic coast (Fig. 1). This basin includes three different types of aquatic ecosystems: a salt-marsh lake (Alimini Grande), a freshwater channel (Zuddeo) and a freshwater lake (Alimini Piccolo or Fontanelle). Alimini Grande receives three main freshwater inputs: one from Alimini Piccolo, a second from a small stream (Zuddeo) and another from three small channels entering the north side of the lake, carrying water from the Traugnano Swamp.

Sampling

The study was performed using leaves of *P. australis* (Cav) Trin. ex Steudel, and was carried out at a total of 20 sampling sites: nine in Alimini Grande, five in Alimini Piccolo and six in the Zuddeo channel. The field work was carried out in four seasonal periods, and datasets on detritus decomposition were collected at four times from the start of the experiment in each season. Detritus processing of *P. australis* leaves was studied using the leaf pack technique, based on the estimate of mass loss from litterbags of reed leaves. For more details see Methods.

Results **Inter-habitat variation**

Overall, *P. australis* processing in the Alimini complex fits a negative exponential model ($y=89.6e^{-0.018x}$; $r=0.700$; $d.f.=16$; $P<0.05$). Considering all sampling stations in both lotic and lentic ecosystems, we calculated an average daily reed detritus weight loss of 1.75% and a reed detritus half-life of 39 d.

Ecosystem types and seasons were sources of heterogeneity, and spatial variation of reed decay rates among ecosystem types was significantly more important than temporal variation (two-way ANOVA, $P < 0.001$) (Tab. 1). Reed leaves decayed faster in the Zuddeo channel ($k=0.036$) than in the two lentic ecosystems, Alimini Piccolo ($k=0.010$) and Alimini Grande ($k=0.007$) (ANCOVA, $F(2,12)=5.9$, $P < 0.05$). The remaining leaf pack biomass at the end of the field experiments (day 90) was $5.6 \pm 8.1\%$ in the lotic ecosystem (Zuddeo), $39.5 \pm 2.5\%$ in Alimini Piccolo and $47.2 \pm 4.7\%$ in Alimini Grande.

On a temporal scale, the processing rates of *P. australis* leaves varied significantly among seasons in both lentic ecosystems, Alimini Grande and Alimini Piccolo (one-way ANOVA, $P < 0.001$), while in the lotic ecosystem, reed processing rates were not affected by seasonal differences. In Lake Alimini Piccolo and Lake Alimini Grande, reed leaf decomposition was 1.3–1.5 times faster in summer than in the other seasons, respectively.

Table 1 - Two-way ANOVA results for ecosystems and seasonal periods of decay-rate data in the drainage basin of Alimini.

Two-way ANOVA				
Source	Sum of Squares	d.f.	Mean Squares	F
Systems	0.070	2	0.035	59.904**
Time	0.001	3	0.000	0.850
Systems*Time	0.006	6	0.001	1.582
Error	0.040	68	0.001	

Intra-habitat variation

Reed detritus decay rates also showed patterns of spatial variation within each ecosystem type. Temporal variance of reed decay rates explained 65% of the overall variance in Alimini Grande, 79% in Alimini Piccolo but only 14% in Zuddeo.

Spatial heterogeneity of reed decomposition rates, expressed as the coefficient of variation among sampling stations, was higher in the freshwater stream (c.v.=61%) than in the freshwater lake (38%) and the salt-marsh lake (33%).

Taking account of the structural features and abiotic characteristics of the ecosystems (Tab. 2), environmental variables explained 74% of variance of leaf decay rates in Alimini Grande ($r=0.858$; d.f.=8, 26; $P<0.001$) and 93% in Alimini Piccolo ($r=0.963$; d.f.=7, 12; $P<0.001$; Tab. 3).

When data were pooled on an annual basis, most of the decay rate variation was found to be due to physical features both in Alimini Piccolo (77%) and in Alimini Grande (45%). On the other hand, when data were analyzed on a seasonal basis, most of the decay rate variation was due to chemical factors, which accounted for up to 93% and 85% of decay rate variance in Alimini Grande and Alimini Piccolo, respectively.

Station	Temp. (°C)	Sal. (PSU)	DO (mg/l)	NH ₃ (µM)	NO ₂ (µM)	NO ₃ (µM)	PO ₄ (µM)	Cl _a (µg/l)
1°	18	29.4	8.2	8.03	0.61	55.76	0.13	2.60
2°	18	28.3	8.1	8.61	0.63	47.51	1.49	3.53
3°	19	21.0	7.5	14.01	0.88	55.28	0.10	2.57
4°	14	10.0	7.6	14.90	1.25	70.69	0.15	3.24
5°	19	30.6	7.5	6.99	0.61	50.06	0.10	3.36
6°	18	30.2	7.7	8.82	0.67	49.34	0.05	3.62
7°	19	29.0	7.1	10.46	0.65	56.10	0.12	4.09
8°	19	28.0	9.8	10.50	0.65	78.27	0.06	3.83
9°	18	31.0	9.3	9.68	0.70	49.54	0.03	3.73
10 ^{oo}	18	0.3	7.7	2.23	0.16	38.89	0.10	20.84
11 ^{oo}	18	0.4	7.7	0.55	0.14	27.29	0.16	32.66
12 ^{oo}	18	0.5	8.3	7.89	0.47	58.07	0.08	13.40
13 ^{oo}	18	0.4	7.7	1.95	0.23	29.94	0.14	33.04
14 ^{oo}	16	0.5	8.3	1.88	0.23	38.28	0.10	32.52
15 ^{ooo}	17	0.2	9.1	1.18	0.26	168.4	0.13	-
16 ^{ooo}	17	0.2	8.3	1.16	0.41	193.0	0.13	-
17 ^{ooo}	18	0.2	7.5	0.72	0.32	226.1	0.14	-
18 ^{ooo}	18	0.3	6.5	0.75	0.23	229.0	0.14	-
19 ^{ooo}	17	0.2	4.8	0.37	0.20	237.0	0.14	-
20 ^{ooo}	17	0.2	4.2	1.14	0.21	211.1	0.09	-

°Alimini Grande, °°Alimini Piccolo, °°°Zuddeo.

Table 2 - Physical-chemical parameters measured at each sampling station in the three ecosystems.

In the lotic ecosystem, the Zuddeo, the distance of each sampling station from the channel mouth in the salt-marsh lake, physical features and nutrients together accounted for 56% of decay rate variance ($r=0.745$; d.f.=7, 16; $P<0.05$; Tab. 3). On an annual

basis, a similar percentage (30%) of decay rate variation was due to structural characteristics and physical features; while on a seasonal basis a pattern was identified only for physical features, which explained 98% of decay-rate variance in summer–autumn and 45% in winter–spring.

Table 3 - Multiple regression analysis of litter breakdown rates in relation to abiotic factors in the lentic and lotic ecosystems.

Multiple regression analysis					
	r	r ²	D.F.	P	S.E.
ALIMINI GRANDE					
All features	0.858	0.736	8, 26	**	0.001
Nutrients	0.809	0.654	5, 29	**	0.001
Physical features	0.668	0.446	3, 31	**	0.002
ALIMINI PICCOLO					
All features	0.963	0.927	7, 12	**	0.001
Nutrients	0.372	0.139	5, 14	n.s.	0.004
Physical features	0.876	0.771	2, 17	**	0.002
ZUDDEO					
All features	0.745	0.555	7, 16	*	0.799
Nutrients	0.319	0.102	4, 19	n.s.	1.043
Physical features	0.544	0.296	2, 21	*	0.878
Structural features	0.543	0.295	1, 22	**	0.858

Discussions and conclusions

The analysis of the results concerning the spatial variability of reed detritus decomposition rates in the Alimini lake complex highlights two important points: **1.** decomposition processes of *P. australis* leaves are characterised by both inter-habitat and intra-habitat heterogeneity; **2.** the structural features and physico–chemical parameters of each ecosystem are factors which influence the spatial heterogeneity of reed processing rates.

The first point is supported, first of all, by evidence that reed decomposition processes are faster in the stream than in the two lakes, and faster in the freshwater than in the brackish lake, in every seasonal period. Here, the three ecosystems investigated have the same climate and natural rock substrate and do not have permanent physical barriers to the dispersion of either invertebrates or micro-fungi, at least between the two freshwater ecosystems. The salinity barrier is broken in the winter period when the salinity of Alimini Grande remains very low along the shoreline between the mouths of the freshwa-

ter stream and the channel connecting it to the freshwater lake (Basset, 2000). Accounting for these structural and physical-chemical features of the Alimini complex, it can be regarded profitably as a natural mesocosm in which there is experimental evidence of differences in detritus decomposition processes between lentic and lotic ecosystems, and between freshwater and brackish ecosystems.

The second point concerns the identification of factors limiting reed processing rates in the two lakes compared with the stream. The results highlight the different importance of structural features and physico-chemical parameters to reed leaf decomposition processes in different ecosystem types. A higher percentage variation of reed decay rates is accounted for by abiotic factors in the two lakes than in the stream. In addition, a seasonal pattern of physico-chemical features and litter breakdown was observed in each ecosystem. The most important difference between lotic and lentic ecosystems is current velocity, which can affect processing rates directly, through leaf fragmentation (Witkamp and Frank, 1969; Hodkinson, 1975; Gurtz and Tate, 1988), and indirectly, by renewing oxygen and favouring microbial activity (Reice, 1974; Godshalk and Wetzel, 1978). The spatial heterogeneity of litter breakdown observed in the Alimini complex cannot be due to the direct influence of current velocity, since average freshwater inflow was low in the Zuddeo ($6.2 \cdot 10^3 \text{ m}^3 \text{ g}^{-1}$), and the leaf packs were protected from fragmentation by the net. Moreover, superficial sediments in both lakes are generally well-oxygenated throughout the year, as is the water of the stream (Basset, 2000).

In conclusion, the present investigation emphasizes the differences in reed decomposition processes in ecosystems of different types and suggests that a complex ecosystem made up of a plurality of ecosystem types, such as the Alimini basin, constitutes an interesting field laboratory in which to test directly

hypotheses concerning factors that limit and regulate detritus processing in aquatic ecosystems.

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