



In situ experimental study of reed leaf decomposition along a full salinity gradient

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ARTICLE INFO

Article history:

Received 3 August 2009

Accepted 18 September 2009

Available online 24 September 2009

Keywords:

Phragmites australis
leaf decomposition
functional indicators
salinity gradient
Ria de Aveiro

ABSTRACT

An experimental study on *Phragmites australis* leaf litter decomposition was conducted in the estuarine environment, Ria de Aveiro, Western Portugal, using the leaf-bag technique, with fine- (1 mm) and coarse-mesh (5 mm) bags. The leaf bags were placed in the field sites at day 0, covering a complete salinity gradient, and replicates were collected over time, at days 3 (leaching), 7, 15, 30 and 60. The biomass loss through the leaching phase, about 20% of the initial leaf mass, was independent of both the salinity and the bag mesh size. The biomass decay pattern along the salinity gradient varied through time and presented strong similarities between the two mesh sizes, with the remaining biomass always lower in the 5 mm mesh-size bags. At days 7 and 15, the lowest remaining biomass was observed at the head of the estuary, the preferential distribution area of *P. australis*. At day 30, the remaining biomass was higher in the marine area and diminished under a direct relationship with salinity, reaching the lowest value in the freshwater environment, with values ranging from 66% to 44% of the initial weight in 5 mm bags, and from 79% to 51% in 1 mm bags. The largest heterogeneity in the remaining biomass among the study areas positioned along the salinity gradient was found close to days 30 (5 mm) and 40 (1 mm). The overall results indicate that the relationship between leaf decay rate and salinity depends on the decay time considered (k_{15} , k_{30} or k_{60}) and, for the later stages (k_{60}), also on the leaf-bag mesh size. This implies that the use of leaf litter decay rates as a functional indicator in transitional waters will need to take into consideration the factor location in the salinity gradient and leaf litter stage at which the decay rate is determined. The differences between the decay rates with the mesh size acted mainly at the level of the absolute k value and not at the level of the pattern along the salinity gradient. Even so, the data obtained at the mouth of the estuary, in the area closest to a fully marine environment, indicated that after the initial biomass loss through leaching, *P. australis* decayed either very slowly, in the 5 mm, or not at all, in the 1 mm mesh bags.

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1. Introduction

The decomposition of organic matter is a key process in the functioning of aquatic ecosystems, enabling the recycling of nutrients and chemical elements, sustaining important food chains and primary production (Takeda and Abe, 2001; Cebrian and Lartigue, 2004). Physical, chemical and biological processes contribute to the decomposition of organic matter, reducing it to elements which are released to the system and made available for uptake by the organisms (Gessner et al., 1999). Internal and external factors contribute to decomposition. Internal factors include namely the leaf species and chemical–physical characteristics of the leaves (Kok et al., 1990; Canhoto and Graça, 1996); the external factors include abiotic, such as environmental descriptors (Webster and

Benfield, 1986) or site characteristics (Sangiorgio et al., 2004), and biotic components, namely microfungi and invertebrates (Graça, 2001; Hieber and Gessner, 2002).

Abiotic and biotic factors affect the process of plant detritus decomposition in aquatic ecosystems at two different levels of hierarchical organisation; the former act mainly as indirect causes of detritus processing rates and efficiencies, while the latter are the direct agents. There is a considerable amount of literature regarding the role of abiotic factors upon litter decomposition rates, namely water temperature (Dang et al., 2009), dissolved nutrients (Bärlocher and Corkum, 2003), oxygen concentration (Chauvet, 1997), and acidity (Thompson and Bärlocher, 1989). Very few works deal with the effect of salinity on decomposition rates (Reice and Herbst, 1982; Christian et al., 1990; Mendelsohn et al., 1999). This is not surprising, since studies on decomposition have been conducted mainly in rivers, streams and lakes, being reported and summarised in a number of books and review papers (Webster and Benfield, 1986; Graça, 2001; Allan and Castillo, 2007), while transitional aquatic ecosystems

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(Twilley et al., 1986; Rossi and Costantini, 2000; Menéndez et al., 2004; Menéndez and Sanmartí, 2007; Sangiorgio et al., 2008), and the marine environment (Harrison and Mann, 1975; Mateo and Romero, 1996), have comparatively received little attention.

Transitional water ecosystems couple continental and marine environments, receiving bio-geochemical inputs from land, rivers and coastal seas, and are important patches in the coastal landscape. They are often reported as extremely productive systems, supporting high rates of primary and secondary production and consequently large quantities of organic matter will be available to decomposition (among others, McLusky and Elliott, 2004). Nevertheless, no single study was found in the literature covering the decomposition process along a full salinity gradient in aquatic environments.

Due to their nature of ecotones between the freshwater and the marine environment, transitional waters show consistent spatial patterns of salinity variation at different temporal scales, from the daily scale of tides to the annual scale of climate patterns. Among transitional water types, rias and estuaries, being river mouth ecosystems, represent ideal model ecosystems to study the influence of water salinity on plant detritus decomposition, since they show a full gradient from marine to freshwater environment within a single ecosystem, maximising the similarity in the chemical environment, preventing barriers to the distribution of organisms and avoiding inverse co-variation of oxygen concentration with salinity, which can characterise other transitional water ecosystems, such as tidal and non tidal lagoons in some Mediterranean areas (see Basset et al., 2006, for a definition of Mediterranean lagoon typology).

This work reports an experimental study on plant detritus decomposition in the estuarine environment, and tests the null hypothesis of no significant differences in the decay rates measured along a salinity gradient ranging from fully marine to freshwater, at different time intervals (days 0–15, days 0–30, days 0–60) and with fine-mesh (1 mm) and coarse-mesh (5 mm) leaf bags, in order to account for the role of larger invertebrates in the decomposition. The study was conducted in Ria de Aveiro, Western Portugal, and the experimental sites were positioned along the Mira Channel, the least stressed in this system by anthropogenic pressure (Castro et al., 2006). Mira Channel offers then the opportunity to estimate the pure effect of the salinity gradient, and associated natural gradients such as dissolved nutrients, occurring in this type of transitional water ecosystems on the process of litter decomposition as expressed by the final result of the process: i.e., the rate of leaf litter decomposition.

2. Materials and methods

2.1. Study area

Ria de Aveiro is a shallow transition system located on Western Portugal, separated from the ocean by a sand bar. Several small river basins meet in Ria de Aveiro, originating an intricate system of narrow channels, islands, intertidal mud and sand flats and salt marsh areas (Fig. 1). The Ria extends along a coastal length of about 45 km and presents a maximum width of 10 km, covering an area of 83 km² and 66 km² at high and low spring tide, respectively. It is characterised by highly variable freshwater discharge and meso-tidal regime (Dias et al., 2000).

Running parallel to the coast and to the South of the entrance channel, the Mira Channel is a shallow 20 km long arm, that receives continuous freshwater input at its head from a small system of ponds and rivers. Salinity conditions in the study area ranged from fully marine, at the mouth, to freshwater, at the head. No studies were ever conducted in Ria de Aveiro in order to estimate the decomposition rate of reed, *Phragmites australis*, or other macrophyte species.

2.2. Field and laboratory procedures

The experimental field work consisted in the follow-up of the biomass decay of *Phragmites australis* leaves, using the leaf-bag technique (Bocock and Gilbert, 1957; Petersen and Cummins, 1974; Melillo et al., 1983). The leaves used in the experiment were collected simultaneously, from the same area, at the end of the 2007 growing season and before they fell on the ground, air-dried and stored in the dark at room temperature and low humidity, until needed. Before use, leaves were cut into 8 cm long fragments excluding the basal and apical parts, oven-dried to constant weight (60 °C for 72 h), and then lots of 3.000 ± 0.005 dry weight were placed in 5 mm and 1 mm mesh bags. The study was performed during winter 2008, in a total of 15 sampling sites distributed in 5 areas (area 1 to area 5), 3 sites per area, along the Mira Channel (Fig. 1). At the beginning of the experiment (day 0), all the leaf bags were placed in the field sites, at the bottom, and replicates were collected over time, at days 3 (for leaching), 7, 15, 30 and day 60. The study was undertaken between January and March. At each sampling time, four replicate leaf bags were collected per site, placed in separate plastic containers, and rapidly brought to the laboratory. Here, the leaves were gently washed to remove sediments and macro-invertebrate colonizers. Leaves from each bag were dried in an oven at 60 °C for 72 h and weighed.

In all areas, at sites A–E (Fig. 1), bottom water samples were taken simultaneously every 30 min during a period of 12 h, totaling 25 samples per site, in order to measure salinity over a tidal cycle. Salinity was expressed using the Practical Salinity Scale that defines salinity as a pure ratio, with no dimensions (UNESCO, 1985). Nutrients (ammonia, phosphates, nitrites and nitrates) were also measured in sites A–E, in bottom water samples taken simultaneously every 3 h covering a period of 9 h. Nutrient concentrations were determined in the laboratory as inorganic dissolved concentrations (Strickland and Parsons, 1972).

Sediment grain size was analysed in all sites where the leaf-bag experiments were conducted, by wet and dry sieving, according to the method described by Quintino et al. (1989): 1) chemical destruction of organic matter with H₂O₂; 2) measurement of the total sediment dry weight, followed by chemical dispersion with tetra-sodium pyrophosphate (30 g/l) and wet sieving through a 63 µm mesh screen; 3) measurement of the second dry weight of the material left on the 63 µm mesh screen and 4) dry sieving of the sand fraction (particles with diameter from 63 µm to 2 mm) and the gravel fraction (particles with diameter above 2 mm), through a battery of sieves spaced at ϕ size intervals ($\phi = -\log_2$ the particle diameter expressed in mm). The silt and clay fraction (fine particles, with diameter below 63 µm) was expressed as a percentage of the total sediment dry weight. These data were used to calculate the median value, corresponding to the diameter that has half the grains finer and half coarser. Given that no detailed grain size analysis was performed for the fine particles, the median could not be calculated for the samples with more than 50% fines content. These sediment samples were classified as mud. Sands (sediments with less than 50% fines) were classified using the median, expressed in ϕ (ϕ) units, according to the Wentworth scale (Doeglas, 1968): very fine sand (median between $3-4\phi$); fine sand ($2-3\phi$); medium sand ($1-2\phi$) or coarse sand ($0-1\phi$).

2.3. Data analysis

The weight loss of *Phragmites australis* was calculated as the fraction of the initial mass, dry weight, remaining at time (t) and expressed as a percent. The decomposition rate was modelled as a negative exponential decay function. This model, proposed by

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