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Fungal biomass and decomposition in *Spartina maritima* leaves in the Mondego salt marsh (Portugal)

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Abstract

Spartina maritima (Curtis) Fernald is a dominant species in the Mondego salt marsh on the western coast of Portugal, and it plays a significant role in estuarine productivity. In this work, leaf litter production dynamics and fungal importance for leaf decomposition processes in *Spartina maritima* were studied. Leaf fall was highly seasonal, being significantly higher during dry months. It ranged from 42 g m^{-2} in June to less than 6 g m^{-2} during the winter. Fungal biomass, measured as ergosterol content, did not differ significantly between standing-decaying leaves and naturally detached leaves. Fungal biomass increased in wet months, with a maximum of 614 μg g^{-1} of ergosterol in January in standing-decaying leaves, and 1077 μg g^{-1} in December, in naturally detached leaves, decreasing greatly in summer. Seasonal pattern of fungal colonization was similar in leaves placed in litterbags on the marsh-sediment surface. However, ergosterol concentrations associated with standing-decaying and naturally detached leaves were always much higher than in litterbagged leaves, suggesting that fungal activity was more important before leaf fall. Dry mass of litterbagged leaves declined rapidly after 1 month (about 50%), mostly due to leaching of soluble organic compounds. After 13 months, *Spartina* leaves had lost 88% of their original dry weight. The decomposition rate constant (k) for *Spartina maritima* leaves was 0.151 $month^{-1}$.

Introduction

Spartina maritima (Curtis) Fernald is a dominant plant species in the upper salt marsh of the Mondego estuary, in the centre of Portugal**.** The importance of *S. maritima* in estuarine productivity, nutrient cycling and biogeochemistry and maintenance of the structural integrity of the salt marsh is likely to be substantial.

It has long been assumed that bacteria are the main agents of plant litter decay in the salt marsh environment and the role of fungi in this process has probably been underestimated. However, research in *Spartina* salt marshes on the Atlantic Coast of North America has shown that microbial production in standing grass litter is dominated by fungi, mainly by ascomycetes. These organisms are important components in the breakdown and use of leaf litter in salt marshes (Newell, 1996). Many species of fungi are associated with *Spartina* spp. For example, Gessner & Kohlmeyer (1976) compilated a list of 101 species of higher filamentous fungi from *Spartina* spp.

Litter decomposition constitutes a key metabolic process in ecosystem dynamics and is strongly regulated by climatic conditions, litter quality, sediment properties and decomposer communities (Valiela et al., 1982; Valiela et al., 1985; Coleman & Crossley, 1996). Information on fungal biomass and activity is critical to assess the importance of these organisms in organic matter processing and trophic dynamics of the ecosystem.

Many factors contribute to the complexity in measuring fungal presence in solid substrates, but the major one is due to the difficulty of isolating fungal hyphae from the substrate (Stahl & Parkin, 1996). Ergosterol

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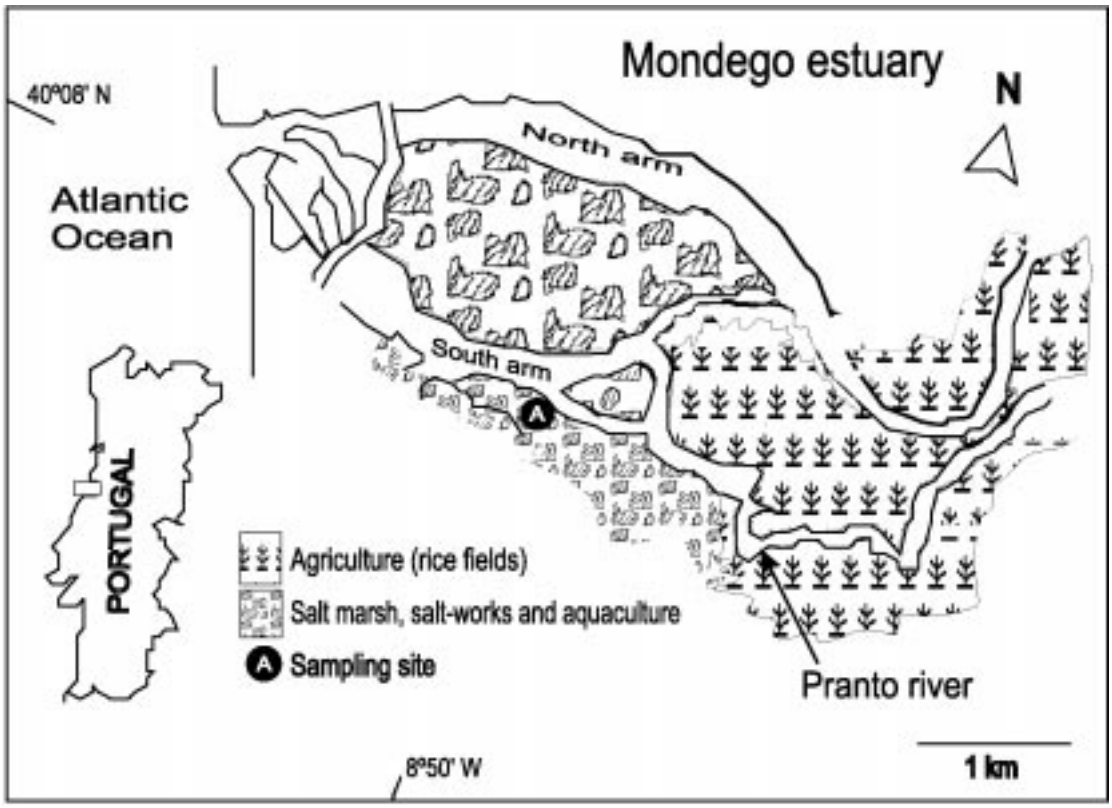

Figure 1. The Mondego estuary.

is a biomolecule, which is largely restricted to the kingdom Fungi and has proven useful as an index of fungal presence (Davis & Lamar, 1992; Newell, 1992).

The main objective of this study was to provide information on fungal importance for leaf senescence and decomposition dynamics of *Spartina maritima* in a Portuguese salt marsh. We compared fungal biomass between two types of leaves before reaching the ground (standing-decaying and naturally detached leaves collected in littertraps) and leaves that were cut, placed in litterbags and allowed to decompose on the marsh sediment.

Materials and methods

Study site

The Mondego estuary is located in the central coast of Portugal (Figure 1) (Marques, 1989). It consists of two arms, north, deeper and south, largely silted-up in the upstream areas, causing the freshwater to flow essentially by the north arm. Consequently, the circulation in south arm depends mainly on tides and small freshwater inputs of the Pranto river. Besides dredging activities related to harbour activities (in the north arm) which cause physical disturbance, this estuary supports several industries, many salt-works and aquaculture farms. It also receives nutrients and chemical discharges from the agriculture areas upstream (Pardal et al., 1993).

Plant material

Standing-decaying leaves (yellow-brown leaves attached to the plant but ready to fall) of *Spartina maritima* (Curtis) Fernald were collected, every month, from February 1996 to January 1997, in a salt marsh ecosystem of the south arm of the Mondego estuary (Figure 1A). A special litter trap was designed to measure leaf litter production (Figure 2). Twenty nylon mesh litter traps, 100 cm long × 40 cm wide, were used. Litter traps were open at the top and naturally detached leaves were collected every month, from March 1996 to January 1997.

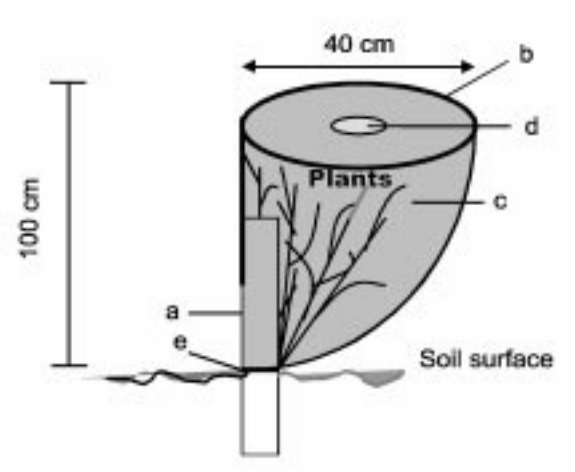

Figure 2. Schematic representation of the litter trap construction: (a) wooden pole; (b) metal hoop; (c) nylon net (4 mm mesh size); (d) opening at the top for litter collection; (e) net closed with a nylon wire around *Spartina* plants.

To study leaf decay, green leaves were collected in August 1996, oven dried at 60 °C (3 g per bag), placed into 24 nylon litterbags (10 cm × 20 cm) with 1 mm mesh size, and allowed to decompose on the sediment surface for 13 months. Considering that the decomposer fungi cannot begin their digestive activities on leaves of *Spartina* spp. before they are dead (Newell & Fallon, 1989), fresh green leaves were collected and senescent process accelerated in laboratory, in order to have the beginning of fungi colonization on the marsh surface. This procedure was adopted to avoid fungal colonization that occurs during leaf senescence. After 1, 3, 5, 7, 9 and 13 months, four bags were randomly sampled. In the laboratory, leaves were rinsed with destilled water and sediment deposits and external plant material removed. Plant subsamples were separated, part to determine fungal biomass and part was oven dried for 2 days at 60 °C to determine dry weight.

Environmental characteristics

In parallel with plant sampling, sediment and water samples were also collected monthly. Samples for measuring pH and salinity were taken near the plant roots during the high water phase of spring tides. Sediments were sampled at approximately 15 cm depth. Salinity was determined by the conductivity method, using a Y.S.I. salinity meter (model 33 SCT), with a precision of 1‰. A Horizon pH meter (model 5995) was used to measure pH. Both pH and salinity measurements were repeated three times. The Portuguese Meteorological Institute provided mean monthly data on air temperature and monthly sums of precipitation.

Fungal biomass

Plant samples collected in the field were rinsed with distilled water to remove adherent clay and dried carefully with filter paper and stored in methanol at −20 °C for periods no longer than 2 months. Ergosterol was extracted from the three types of leaf samples (standing-decaying, naturally detached and litterbagged leaves following the methods described by Newell et al. (1988) and Gessner et al. (1991).

Extraction involving homogenization was carried out in 15 ml of methanol (in an ice bath) for 2 min. Homogenates were centrifuged for 5 min at 450 rpm. Supernatants were collected in a 100 ml round bottom flask containing 1.6 g KOH and 4 ml of ethanol. The pellet was re-extracted with 5 ml of methanol. Primary and secondary supernatants were combined and refluxed for 30 min in a water bath at 80 °C.

The saponified solution was filtered (Whatman GF/C filters) and collected in a 250 ml separatory funnel and 8 ml of distilled water was added. Ergosterol was removed from the alcoholic phase by partitioning into hexane (three 20 ml aliquots of hexane). After each addition and mixing, the upper hexane layer was withdrawn and hexane fractions from each sample were pooled. Hexane fractions were evaporated to about 2 ml with a rotating evaporator (Heidolph VV 2000) in a water bath at 30 °C. These were then transferred to 10 ml tubes and evaporated to complete dryness at 40 °C under a stream of N_2 gas. Samples were dissolved in 2 ml of methanol 'Gradient Grade' (LiChrosolv) and stored at −20 °C for 2 days maximum.

Samples were briefly centrifuged and injected into a Merck Hitachi HPLC consisting of an L-6200A pump, an AS-2000A injector and an L-4250 UV-VIS wavelength absorbance detector. The column was reversed-phase (Lichrosphere ODS2), 25 cm by 4.6 cm with 5 μm packing, with methanol at a flow rate of 2 ml min^{-1}. The concentrations of ergosterol were determined by measuring the absorbance at 282 nm approximately 9 min after injection and comparing with a standard curve using known ergosterol concentrations (Fluka chemicals), (triplicate injection standards).

During the entire procedure, samples were protected from direct sunlight.

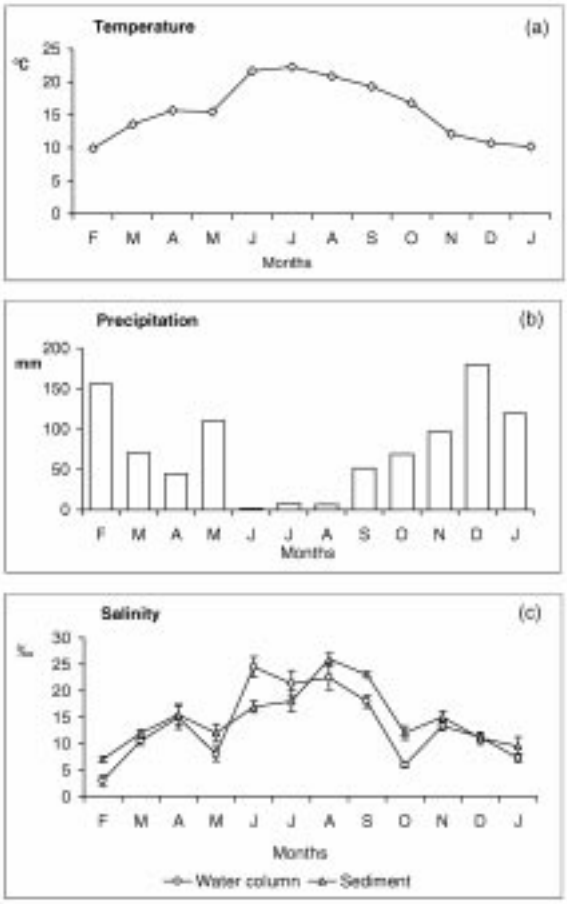

Figure 3. Environmental parameters monthly measured in the Mondego salt marsh, from February 1996 to January 1997.

Statistical analysis

Leaf fall was determined as the sum of naturally detached leaves collected in littertraps every month. The decomposition rate constant k (Wieder & Lang, 1982), was estimated using the first order exponential equation:

$$\ln (X_t / X_0) = -kt,$$

where X_0 is the initial amount of litter, X_t is the litter remaining at a time t. In this study, t represents a month interval. Differences between mean ergosterol content in standing-decaying and detached leaves and seasonal variation of ergosterol concentrations for all types of leaves were tested using ANOVA (Zar, 1996).

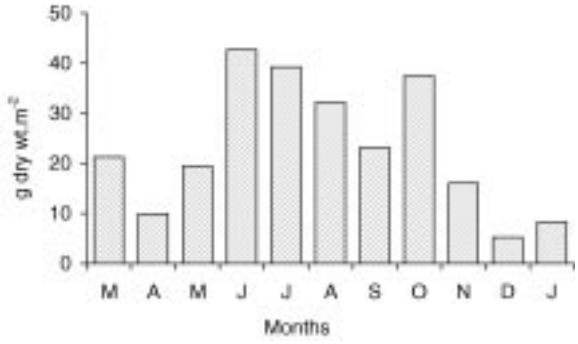

Figure 4. Monthly leaf fall of *Spartina maritima* quantified from March 1996 to January 1997 in the upper Mondego salt marsh.

Results

Environmental parameters

During the summer months, mean monthly air temperature varied from 21.7 °C to 22.3 °C, whereas in winter, temperature declined reaching a minimum of 9.9 °C in February (Figure 3a). During the study period, dry summer months were reported. June was the driest month with 1.5 mm precipitation and December the wettest registered with 180 mm (Figure 3b).

Higher salinity mean values occurred during summer (Figure 3c). In the water column, maximum values were registered in June (24.5‰) and August (22.5‰) and the minimum in February (3‰). Salinity values recorded in the sediment showed a maximum of 26‰ in August and a minimum of 7‰ in February. Mean pH in the sediment was always below 7.1, except in October when it reached 8.1. In the water column, pH varied between 7.3 and 7.9 throughout the year.

Leaf fall

Leaf fall (naturally detached leaves) was significantly higher during dry months and two peaks were clearly observed in summer and in early autumn (Figure 4). Maximum leaf fall (42 g dry wt m^{-2}) was found in June, decreasing steadily to 23 g dry wt m^{-2}in September. The second peak was observed in October (37 g dry wt m^{-2}). During winter, leaf fall was lower, with a minimum (5 g dry wt m^{-2}) observed in December.

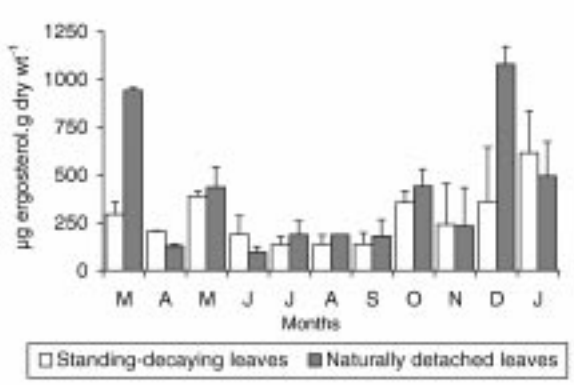

Figure 5. Ergosterol concentrations (mean ± standard deviation) in standing-decaying and naturally detached leaves of *S. maritima* from March 1996 to January 1997.

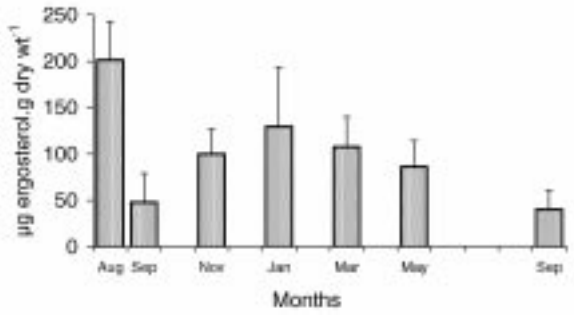

Figure 6. Ergosterol content (mean ± standard deviation) of summer-harvested *S. maritima* leaves decomposing from August 1996 to September 1997 on the sediment surface of the upper Mondego salt marsh.

Fungal biomass

Fungal biomass, measured as ergosterol content, did not differ significantly between standing-decaying leaves and naturally detached leaves ($F_{1,47}$=0.647; P=0.43). March and January showed, however, large differences, with much higher ergosterol values registered in recently detached leaf blades. A seasonal pattern in fungal biomass was noticed in both types of leaves ($P < 0.05$), with higher ergosterol content registered in wet months, decreasing greatly in summer (Figure 5). Naturally detached leaves showed higher ergosterol concentrations in March and December, 939 and 1077 μg g^{-1}, respectively, and low levels during summer (<185 μg g^{-1}). Litterbagged leaves showed a pattern of fungal biomass similar to the one observed in standing-decaying and detached leaves ($P < 0.05$) (Figure 6), with higher values in rainy months. Green leaves harvested in late August had an average of ergosterol content of 201 μg g^{-1}. However, after 1-month of decomposition, a sharp decline was observed (47 μg g^{-1}). Subsequently, ergosterol increased to a maximum of 129 μg g^{-1} in January, and decreased steadily between January and September.

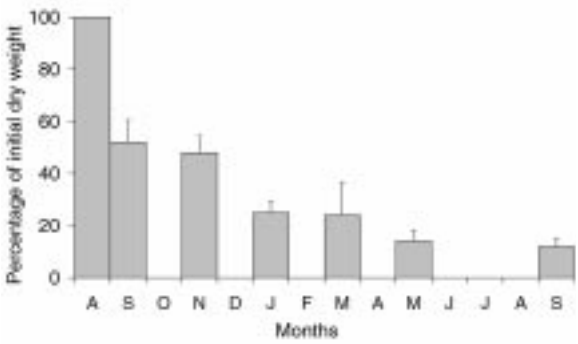

Figure 7. Mass loss of summer-harvested green leaves *Spartina maritima* placed in litter bags on the sediment surface of the upper Mondego salt marsh (k= decomposition constant).

Leaf decomposition on the marsh sediment

Dry weight declined rapidly during the first month and thereafter continued to slowly decline (Figure 7). About 48% of the original biomass was lost after the first month. At the end of the study period (13 months), *Spartina* leaves had lost 88% of their original dry weight. The decomposition rate constant value (k) for *Spartina maritima* leaves was 0.151 month^{-1} (R^2 = 76.2%; SE = 0.33).

Discussion

Seasonality of leaf fall was evident in *S. maritima* (Figure 4), with maximum values associated with dry summer months. This could be a response to water stress. In fact, low rainfall and high evaporation leads to higher soil salinities (Figure 3). Increased salinization and high evapotranspiration rates in dry months tend to decrease sediment water potential, making it more difficult for plants to maintain osmotic adjustment (Rozema et al., 1985). Transpiration thus becomes metabolically more expensive (Wium-Andersen, 1981). The higher leaf fall rate observed in dry periods could also be due to an increased fragility of the decaying blades that had been weakened by fungal decay during wet months. Higher ergosterol concentrations were observed in cold months (Figure 5), indicating that fungal activity is more intense in winter. Summer leaves suffer higher decomposition

and, thus, are more likely to fall. With increasing air temperature and dry weather, standing-decaying blades of *S. maritima* become much less flexible and more fragile (S. Y. Newell, pers. comm.).

A significant seasonal effect on fungal biomass was recorded for leaves decomposing attached to the plant and naturally detached leaves (Figure 5), with higher values determined in leaves collected in winter. The same pattern was observed in leaves decomposing on the marsh sediment (Figure 6). Leaf decay in the salt marsh is subjected to irregular shifting from water sufficiency to water stress (Newell et al., 1991) and tissue water content is known to influence decay rates (Newell et al., 1985; Kuehn & Suberkropp, 1998a). In summer, higher air temperatures and higher salinity values, provide less favourable moisture conditions for fungal survival in plant substrate.

In the present study, no significant differences in ergosterol concentrations were observed between standing-decaying and naturally detached leaves, suggesting that, once established during senescence, fungal decomposers do not change greatly their biomass concentration (Kuehn & Suberkropp, 1998b), until leaves reach the marsh sediment (Figure 6). Samiaji & Bärlocher (1996) also observed that litterbagged leaves of *Spartina alterniflora* had lower ergosterol concentrations (2–4 times lower) than standing-dead leaves. They also found that leaves, before reaching the sediment, carried more fungal species and lower bacteria values than leaves in litter bags. Newell & Fallon (1989) demonstrated that bacteria concentrations were much higher in tagged standing leaves than in bagged leaves of cordgrass. Considering these results and also results from Gessner & Goos (1973) and Newell et al. (1996), it is most likely that, as in North American salt marshes, fungi that decompose *S. maritima* leaves in the Mondego salt marsh, are replaced by bacteria in this process, when leaves fall and reach the sediments. Xiong & Nilsson (1997) speculate that fungi and bacteria probably shift their relative abundances in riparian areas following the fluctuations between wet and dry conditions. Newell (1996) suggests that high fungal yields are possible in standing-decaying *Spartina* leaves, since very efficient capture of fungal-lysed plant macromolecules might occur within the maze of hyphal channels inside decaying leaves, reducing amounts of lysates that may leach away.

Biotic action (macrofauna, mesofauna and microfauna) on substrate quality together with climate conditions are the two main factors controlling rates of litter decomposition (Coleman & Crossley, 1996). The mesh bags used in the present study (1 mm) excluded most macrofauna from the decomposition action, because our aim was to study fungal action on this process. This technique, however, may underestimate the rate of decomposition because the litter bags restrict the entry of large detritivorous (Newell, 1996). Dry weight remaining in the bags was severely reduced by 50% after the initial month (Figure 7). The decay rate was very high initially, probably due to almost immediate microbial uptake and decomposition of simple organic compounds and leaching (Valiela, 1985) or, it could be, to some extent, an artifact resulting from predrying of the leaves as referred by Samiaji & Bärlocher (1996).

We expected to find low ergosterol content in green leaves; however, near 200 ug g^{-1} were observed. The choice of green substrate, predrying plant samples and the use of litter bags, eventually created artifact conditions, as explained by Newell (1993). Newell & Fallon (1989) also reported that the use of litter bags gave artifactitious results not reflecting the natural standing-decay process. However, after 1 month in the sediment surface, ergosterol concentration decreased sharply to a more realistic value of 47 ug g^{-1}.

Our finding of 200 μg ergosterol g^{-1} in green leaves might also be due to a misidentified peak, which eluted from the HPLC column at a similar retention time as had been found by like Newell & Fell (1992) for mangrove leaves. They observed a neutral-lipid substance with 282-nm absorbance and a retention time relative to ergosterol of 0.9.

In summary, this work showed that higher fungal biomass rates in decomposing leaves occur in wet months and provided evidence that, as observed in studies with cordgrass in North American salt marshes, fungal activity seems to be more important for leaf decay of *Spartina maritima* when leaves are still attached to the plant in European marshes.

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