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JOÃO CARLOS MARQUES
MANUEL AUGUSTO GRAÇA
Scientific Editors

Aquatic Ecology of the Mondego River Basin
Global Importance of Local Experience

Coimbra • Imprensa da Universidade

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NITROGEN DYNAMICS IN THE MONDEGO ESTUARY: LEAF SENESCENCE AND N MOBILISATION IN *Spartina maritima*

Abstract

Spartina maritima (Curtis) Fernald is a dominant species in the Mondego salt marsh playing a significant role in estuarine productivity and energy cycling. A study conducted between February 1996 and January 1997 revealed that nitrate was mainly transported from roots and rhizomes to the leaves where it was accumulated. Nitrate concentration increased during summer in all types of studied leaves, probably playing an important role in osmotic regulation. Ammonium, on the contrary, was incorporated into organic compounds in the root rather than transported into the leaves. Total nitrogen quantified in the leaves, showed an obvious pattern, decreasing greatly during summer and early autumn. From March to June, nitrogen content in green leaves decreased nearly 75 %. This variation of nitrogen content was highly correlated with its concentration in the sediment. Leaf ageing in *Spartina maritima* was associated to chlorophyll losses, to nitrate and total nitrogen remobilization, and also to ammonium accumulation.

Introduction

In salt marshes, nitrogen supply is mediated by redox of the sediments and limits plant growth (Valiela 1995). Nitrate and ammonium are the major sources of inorganic nitrogen taken up by roots of *Spartina alterniflora* as well as in general higher plants (Wirén et al. 1997). Nitrate is readily mobilised in xylem and can be stored in vacuoles of roots, shoots, and storage organs (Martinoia et al. 1981). Its accumulation in vacuoles is of considerable importance for cation-anion balance and osmoregulation (Smirnoff and Stewart 1985). Ammonium is incorporated into organic compounds and generally not stored in cells (Schortemeyer et al. 1997).

Spartina maritima is a dominant species in the Mondego salt marsh (Portugal) and plays an important role in nutrient and energy dynamics in European salt marshes similar to that played by *Spartina alterniflora* in North American marshes. Like most

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coastal producers, throughout the world *Spartina maritima* is subject to eutrophication, which elevates nitrogen levels in the sediment (Valiela et al. 1992). Increased nitrogen supply increases growth and biomass, and affects plant morphology, nutrient content, density and reproduction (Rogers et al. 1998).

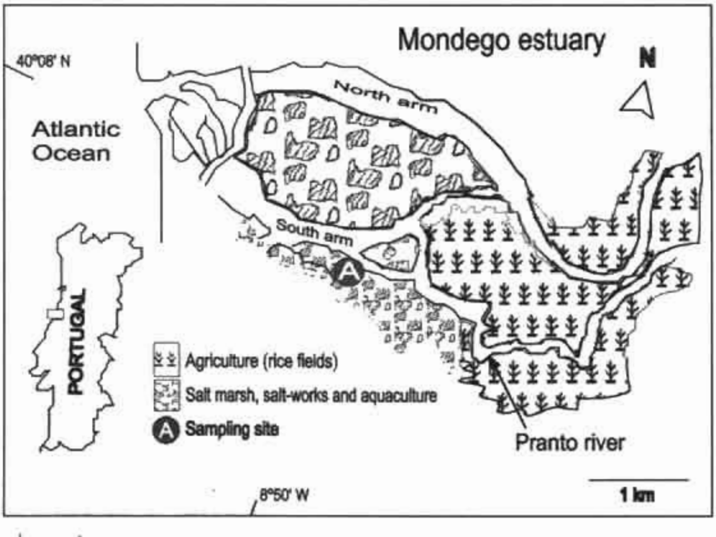
In general, plant leaves undergo a series of developmental changes. Initially, leaves are predominantly heterotrophic organs, maturing to become a net exporter of photosynthates. After reaching maturity, the leaf exhibits a reduced capacity to act as a source of photosynthetically fixed carbon and enters a senescent phase (Turgeon 1989). Leaf senescence represents a key developmental phase in plant life, which is as ordered and complex as any other phase of development. During leaf senescence, N, C and minerals are mobilised from the mature leaf to other parts of the plant. This process involves a series of events like cessation of photosynthesis, disintegration of chloroplasts, breakdown of proteins, loss of chlorophyll and removal of amino acids (Buchanan-Wollaston 1997).

The main objective of this work was to estimate seagrass contribution to the nitrogen dynamics in a Portuguese saltmarsh. This paper reports the seasonal variation of inorganic and total nitrogen concentrations in the Mondego saltmarsh and *Spartina maritima* contribution to this process. Nitrogen mobilisation during leaf senescence and its importance for plant survival in this system was also studied.

Material and methods

Study site

This work was conducted at the Mondego estuary located on the western coast of Portugal (40°08' N / 8°50' W) (Fig. 1). The Mondego estuary consists of two arms



1. The Mondego estuary.

with different hydrographic characteristics. The northern arm is deeper, while the southern arm is largely silted up, routing most of the freshwater input through the northern arm. Water circulation in the southern arm is mainly dependent on tidal cycles, receiving also small freshwater inputs of the Pranto river, a tributary with large amounts of nitrogen substances, frequently used as fertilisers by rice farmers in upstream areas (Marques 1989, Flindt et al. 1997). Sampling site was located on the south arm, in a salt marsh dominated by *Spartina maritima* (Fig. 1A).

Field program and laboratory procedures

Spartina maritima plants were sampled monthly from February 1996 to January 1997. In the laboratory, biomass samples were separated into roots, rhizomes, and leaves. Leaves were separated in 3 leaf age groups: green leaves (GL) – live tissue with green colour; senescent leaves (SL1 and SL2) – senescent tissue with at least 1/3 of area yellow (SL1), leaves with almost 2/3 of their area with yellow-brown color (SL2) and standing-decaying leaves (SDL) – yellow-brown dead material ready to fall. Leaves detached via decay were collected using 20 nylon mesh litter traps 100 cm long x 40 cm wide, opened at the top for monthly collections (Castro and Freitas 2000), designated in this paper as litter trap leaves (LTL). Samples were weighed and dried at 60°C until constant weight. Dry tissues were ground and analysed for NO_3^- by the acid salicylic method (Cataldo et al. 1975), for NH_4^+ by the indophenol blue method (Solorzano 1969), and for total N (only in leaf samples) by the Kjeldhal method (Bremner and Mulvney 1982).

Immediately after collection, the chlorophyll (a + b) content was measured spectrophotometrically on triplicate 300 mg (fresh weight) of leaf samples (GL, SL1, SL2 and SDL), after extraction with 80 % acetone (Henry and Price 1993).

Sediment samples were collected each month in parallel to plant samples. Sediments were oven-dried at 60°C for 3 days and stored in sterilised plastic bags. Total N was determined in 5 g of sediment following the method in Bonneau and Souchier (1979).

Statistical analysis

One Way Analysis o Variance (ANOVA) was used to test for differences between mean nitrogen concentrations in plant samples, chlorophyll content in leaves, and seasonal variation of these variables. Non-parametric analysis of variance (Kruskal-Wallis) was used whenever parametric assumptions were not verified (Zar 1996).

Results

Water content

Water content (percentage of fresh weight) was higher in belowground tissues than in leaves (Fig. 2). Water content was relatively constant in roots and rhizomes, with higher values in root tissues. Rhizomes showed a medium annual value of 76 % of water and roots of 84 %. Young leaves were more succulent than senescent and dead leaves. All types of leaves revealed a decrease in water content from February to April, increasing the month after. Green and senescent leaves showed a slight decrease during summer. Water content in standing dead leaves increased from May to October, remaining high in winter.

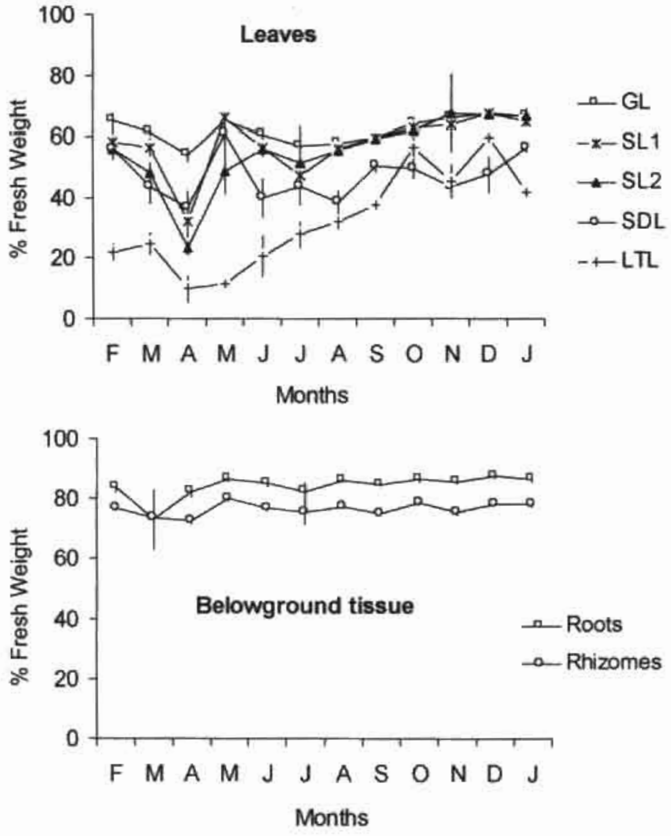


Fig. 2. Water content, expressed as percentage of fresh weight, in *Spartina maritima* leaves, roots and rhizomes, collected from February 1996 to January 1997.

Chlorophyll

Chlorophyll levels were different between the four types of leaves ($H_{3,N=144}=123$; $P<0.001$; Kruskal-Wallis ANOVA), decreasing with leaf senescence (Fig. 3). Overall, mean chlorophyll concentration for GL was 24.5 ± 3.1 mg L^{-1}. Mean losses of 17 %, 39 % and 58 % were measured for SL1, SL2 and SDL, respectively. Although each type of leaf followed its own seasonal change, a late-summer decrease and autumn increase were common features of the annual pattern.

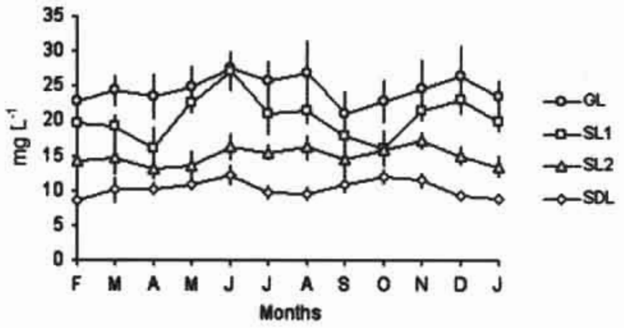


Fig. 3. Chlorophyll content (a + b) in leaves of *Spartina maritima*, monthly collected from February 1996 to January 1997.

Nitrogen dynamics

Nitrate was clearly transported and accumulated in young leaves (Fig. 4). During the study period, belowground roots and rhizomes showed levels of NO_3^- 4-5 times lower than GL. Seasonal variations were observed in all types of leaves, with higher

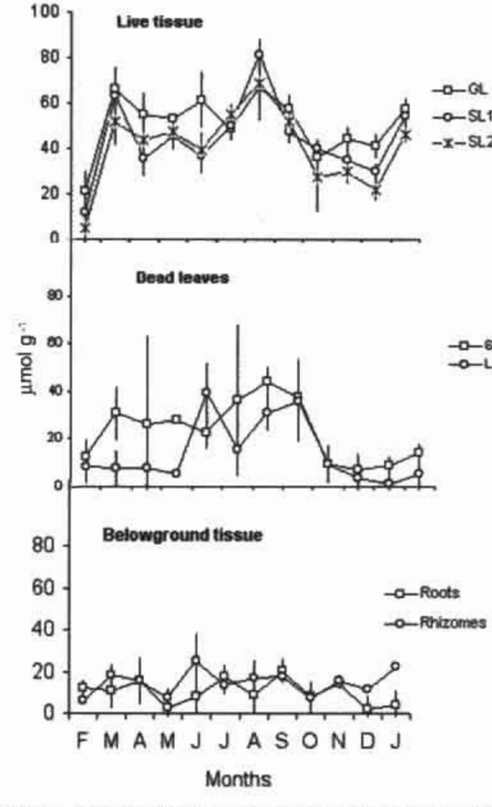


Fig. 4. Nitrate concentrations determined in plant samples between February 1996 and January 1997.

values in summer months. Differences between root and rhizome NO_3^- concentrations were verified ($F_{1,219}$=7.46; P<0,01). Seasonal variations for belowground tissues were also observed with slight increase in dry months. NO_3^- mean concentrations in rhizomes ranged from 6.4 ± 1.7 µmol g^{-1} dry wt (February) to 25.0 ± 12.8 µmol g^{-1} dry wt (June). Nitrate leaf content changed greatly with leaf age ($F_{4,374}$=49.78; P<0,001). Higher concentrations consistently occurred in the youngest age class, with large decrement from mature to senescent leaves. Leaves detached via decay (LTL) revealed about 70 % less nitrate than GL.

Mean monthly concentrations of NH_4^+ increased with leaf senescence ($F_{4,175}$ = 14.35; P<0,05). Like NO_3^-, all types of leaves showed NH_4^+ seasonal changes (Fig. 5). A slight decrease in NH_4^+ content was observed in leaves collected during summer. Standing-decaying leaves (SDL) showed ammonium concentrations 2-3 times higher than GL. Similar concentrations were found in belowground tissues, and roots and rhizomes did not show statistical differences in their values ($F_{1,70}$ = 0.52; P=0.47).

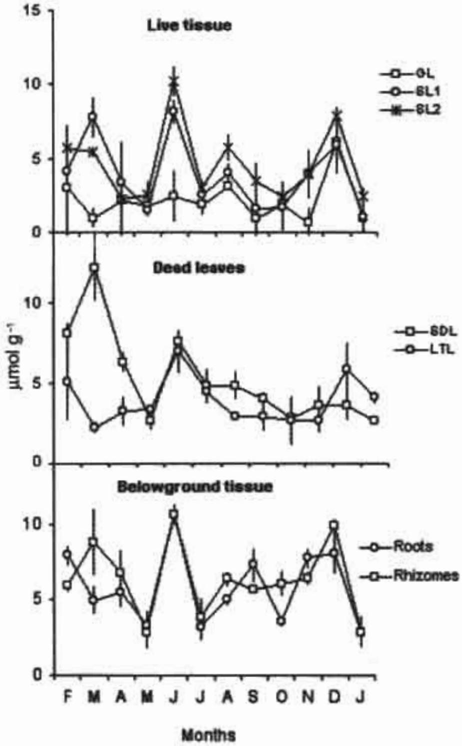


Fig. 5. Ammonium concentrations determined in plant samples between February 1996 and January 1997.

All leaves revealed a strong seasonal variation, with leaves produced in early spring and winter showing higher N levels (Fig. 6). Total nitrogen content (TN) was significantly different between the types of leaves analysed ($F_{4,155}$ = 6.94; P<0.05). Mean N concentrations in GL and SL1 were generally higher from that observed in older leaves from February to June. This difference decreased and was even inverted during summer and autumn, recovering in the last month.

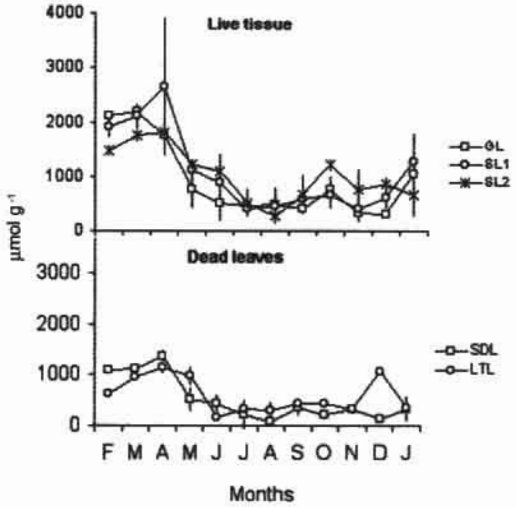


Fig. 6. Total nitrogen content measured in leaves of *Spartina maritima* between February 1996 and January 1997.

Total nitrogen (TN) concentrations in the sediment were much higher in spring and winter (Fig. 7). Maximum value of 20 ± 0.5 mmol g^{-1} dry wt was measured in April. In June, total nitrogen concentrations decreased to 5 ± 1.1 mmol g^{-1} dry wt.

Fig. 7. Seasonal changes in nitrogen concentrations in sediment samples, collected in the Mondego salt marsh from February 1996 to January 1997.

Discussion

Nitrate translocation from roots to leaves (Fig. 4) can be explained by the need of tissue osmotic adjustment, particularly important during summer, when soil salinity increases (Flowers et al. 1986). The accumulation of salts participate in this function and is the least energetic costly mechanism of osmolarity generation (Raven 1985). Water content in *Spartina maritima* leaves did not change greatly along the year in living leaves, suggesting that succulence is not an important strategy for tissue osmoregulation (Fig. 2). Considering that, contrarily to dicotiledone plants (Flowers et al. 1986), grasses do not use succulence as a major adaptation to soil salinity, nitrate can thus be considered to play an important role in this process for this species. Our results, particularly the NO_3^- accumulation in summer leaves, showed that NO_3^- can also work as an important mechanism to lower the energy costs of the plant for nitrate reduction and assimilation in these tissues, since the energy can be directly given by the photosynthesis process (Abrol et al. 1983), and also decreasing the production of toxic oxygen radicals. Several studies demonstrate that light increases nitrate metabolism rates (Delhon et al. 1995, Keller et al. 1995, Cárdenas-Navarro et al. 1998).

Toxic ammonium effects are well known in terrestrial plants (Gerendás et al. 1997, Pearson and Stewart 1993), but for marine environments we can expect to have higher NH_4^+ concentrations in plant tissues, since salt concentration in sediments is much higher, and plants are probably more tolerant to this nutrient. However, this was not observed. Whereas NO_3^- was stored in leaves without detrimental effect, NH_4^+ content was quite low in both aerial and subterranean organs. This showed that, despite the high NH_4^+ concentrations in the soil (Fig. 5), *Spartina maritima* has several physiological mechanisms to prevent the increase of this nutrient in its tissues. Van Katwijk et al (1997) showed that the presence of concentrations as low as 25 μM of NH_4^+ in sediments were toxic to Zostera marina plants. Fan et al. (1997) also suggested that the use of NO_3^- instead of NH_4^+ as an N source is important for rice plants to tolerate anaerobiosis conditions of the soil, particularly for seed germination, because it gives a more efficient economy of energy and minimises lower pH effects in cells cytoplasm.

The lower N concentrations observed in summer were expected because the flowering period of this species occurs between May and July, and nutrients become thus necessary for plant reproduction (Marschner 1995). What is interesting is that the N seasonal variation in the soil corresponds almost exactly to the same variation in the sediment (Fig. 7) reflecting an important strategy that *Spartina maritima* plants use to improve nutritional efficiency during low external N availability (Thornton et al. 1994). During summer a decline of N absorption at root level is expected (Correia et al. 1992), mainly due to the reduction of carbohydrate levels in root cells (Marschner 1995).

Ammonium concentrations have been shown to increase during leaf senescence for several species (e.g. Thomas 1978, Peters and Van Laere 1992, Chen et al. 1997). This study showed that it also happens in *Spartina maritima* (Fig. 5). A decrease in glutamine synthetase activity, breakdown of macromolecules (e.g., proteins and nucleic acids) and an increase in nitrate reduction during leaf ageing, are some of the factors controlling ammonium accumulation (Storey and Beevers 1978, Platt and Anthon

1981, Kar and Feierabend 1984, Chen and Kao 1998). Chen et al. (1997) suggested that NH_4^+ could participate in senescence regulation of rice leaves, with the argument that increases in ammonium concentrations could make foliar tissues more sensitive to the action of ethylene, thus promoting senescence.

An opposite pattern was observed for nitrate and total N; senescent leaves clearly showed lower nitrate concentrations than green leaves. Castro and Freitas (2000) showed that higher leaf fall rates of *Spartina maritima* occur during summer, peaking in July and that these results are clearly related to the decrease of N and NO_3^- in leaves before they fall, contributing to nutrient and energy saving of the plant (Marschner 1995, Hayati et al. 1995).

Conclusions

Leaf senescence and decomposition are important processes for the cycling of nutrients in the salt marsh system (Cornelissen 1996, Rutigliano et al. 1996, Aerts and Caluwe 1997, Mugendi and Nair 1997). Nutrient regulation, particularly nitrogen, is a very important process during plant development (Marschner et al. 1997). This work showed that *Spartina maritima* was capable of adjusting N distribution when growing in low N conditions and also before leaf fall in order to avoid the waste of N and energy, indispensable for plant survival in salt marsh systems. Nitrate and total N content decreased greatly with leaf senescing and before major leaf fall occurred. NH_4^+ was not accumulated in plant cells, and it's concentration increased with leaf ageing, resulting from the normal process of senescence, but most likely to have an important role in controlling such process.

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