

The response of primary producer assemblages to mitigation measures to reduce eutrophication in a temperate estuary

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Abstract

The Mondego estuary is a well-described system located on the North Atlantic Ocean, where cultural eutrophication progressed over the last decades of the 20th century. Consequently, and due to a large productivity of *Ulva* spp., *Zostera noltii* meadows were severely reduced with a concomitant decrease in environmental quality. In 1998, experimental mitigation measures were implemented, via changes in hydrology to increase circulation and diversion of nutrient-rich freshwater inflow, to reverse the process in the most affected area of the estuary – its South arm.

The objective of this study was to assess the differences in response of primary producer assemblages to the implemented measures to reduce eutrophication.

Results show that the mean concentrations of DIN suffered a notorious decrease due to a significant reduction in the ammonium concentration in the water column, while DIP increased significantly. Primary producer assemblages showed different responses to these changes: phytoplankton, measured as concentration of chlorophyll *a*, did not show any significant changes; green macroalgae, mostly *Ulva* spp., suffered a large reduction in biomass, whereas *Gracilaria gracilis* and the macrophyte *Zostera noltii* biomasses increased greatly. Results show that phytoplankton biomass has remained constant and suggest that the reduction in ammonium could have been responsible for the changes in the green macroalgal biomass. Light was the most likely factor in the response of seagrass whereas red macroalgal reaction seemed to be dependent on both light and ammonium.

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1. Introduction

In estuarine systems, plant communities are constituted by complex assemblages of phytobenthos and phytoplankton, each with different access to nutrients and light (Taylor et al., 1995) that can constitute potentially limiting factors to the primary production of these aquatic autotrophs (Pedersen and Borum, 1992). Phytoplankton and fast-growing ephemeral macroalgae are often limited by nutrient availability, while slow-growing perennial macroalgae and rooted macrophytes

seem less dependent on nutrient concentrations (Sand-Jensen and Borum, 1991).

In the last decades, anthropogenic activities have enhanced the enrichment of water bodies with nutrients, particularly nitrogen and phosphorus, named as “cultural eutrophication”. Agricultural run-off, waste discharges from industries and fish farms amongst others are responsible for nutrient inputs into aquatic systems (Menéndez and Comín, 2000; Hernández et al., 2002; Nedwell et al., 2002).

Phytoplankton and macroalgae are capable of taking advantage of the available resources in transient environments (Viaroli et al., 1996; Raven and Taylor, 2003; Cohen and Fong, 2004). Their high surface area to volume ratio and high affinity for nutrients, especially N and P, favor a rapid

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nutrient uptake and high growth and production rates leading to very large biomass values (Rosenberg and Ramus, 1984; Hernández et al., 1997; Raffaelli et al., 1998; Raven and Taylor, 2003). Among the macroalgal species found in areas undergoing eutrophication are the genus *Chaetomorpha*, *Cladophora*, *Gracilaria* and *Ulva* (Raffaelli et al., 1998; Mistri et al., 2001; Fong et al., 2004). By influencing benthic nutrient processes through interception of light and water column nutrients (Boyer and Fong, 2005), they often out-compete other species, usually late-successional, long-lived species like perennial macroalgae (e.g. *Fucus*) and seagrass (e.g. *Zostera*) (Peckol and Rivers, 1996; Menéndez and Comín, 2000).

Seagrass are important primary producers in estuarine systems and their abundance and distribution are strongly correlated with light availability (Kraemer and Hanisak, 2000). Eutrophication effects on seagrass meadows are stronger in sheltered environments with frequent and high nutrient loadings, reduced tidal flushing and fluctuating temperatures (Maier and Pregnall, 1990). Increased nitrogen loading has been pointed out as an important cause of seagrass loss by stimulating competition for available light (e.g. van Katwijk et al., 1997; Brun et al., 2002; Valiela and Bowen, 2007).

Due to the unique importance of seagrass meadows in the ecosystems, it is necessary to take measures to minimize and revert the effects of eutrophication, bringing the systems into the previous stable state (e.g. Webster and Harris, 2004). However, to guarantee that the restoration programmes are successful, it is important to understand the mechanisms that have led to the ecological changes (Zhang et al., 2003). In the case of macroalgal blooms, the knowledge of their responses to changes in their driving variables (e.g. nutrient loadings, hydrodynamics) is essential to understand the way the system will react and thus assuring its recovery (e.g. Webster and Harris, 2004).

The Mondego estuary is a temperate, intertidal ecosystem that has been for the last decades under ecological stress caused mainly by eutrophication. Overall the system presented itself with a severe decrease in environmental quality (Lillebø et al., 2007; Teixeira et al., 2007), and to revert this condition, in 1998, a management plan was implemented with measures that included the reduction of nutrient load to the system South arm, the increase in hydrodynamics in order to reduce the water residence time and the physical protection of the seagrass meadows (for further information see Lillebø et al., 2005).

The aim of the present study was to assess the response of phytoplankton (accessed as concentration of chlorophyll *a*), the macroalgae *Ulva* spp. and *Gracilaria gracilis* and the seagrass *Zostera noltii* (Hornem) to the mitigation measures implemented in the Mondego estuary to reduce the eutrophication symptoms.

2. Material and methods

2.1. Study area

The Mondego estuary is located on the Western Coast of Portugal (40°08'N; 8°50'W), with an approximate area of

1072 ha and 7 km long, characterized by a temperate coastal climate with Mediterranean and Atlantic influences. It comprises two arms, North and South, separated by an alluvium-formed island (Murraceira Island) that joins again near the mouth. The North arm of the sea is deeper (4–8 m during high tide, tidal range 1–3 m), while the South arm is shallower (2–4 m during high tide, tidal range 1–3 m) and until 1998 it was largely silted up in the upstream areas, which caused freshwater to flow mainly through the North arm. As a consequence, water circulation was dependent on tides and freshwater discharges (which constituted an important input of nutrients) from a small tributary, the Pranto River (Fig. 1).

In 1998, a restoration program was implemented to reverse the process of eutrophication in the most affected area of the estuary – the South arm (Fig. 1), comprising several measures. To reduce the loadings of nutrients into the South arm from the Pranto River the sluice aperture was reduced and most of the freshwater flow from this tributary was diverted to the North arm by another sluice located upstream. To improve water circulation the connection between both arms was enlarged allowing water to flow from the North arms during high tide. The remainder of the seagrass patches was delimited by wooden stakes to prevent physical disturbance and awareness meetings were held to inform the population about the importance of these areas (for more detailed description see Lillebø et al., 2005, 2007).

The summary of the main characteristics of the South arm of the estuary is presented in Table 1.

2.2. Field program and laboratory procedures

The study was conducted between February 1993 and December 2004 in the South arm of the Mondego estuary, as a part of a long-term monitoring program. Three sites (*a*, *b* and *c* – Fig. 1A) were selected based on macroalgal abundance following a preoperational gradient increasing from downstream to upstream. The distance between sites *a* and *b* is 0.25 km and between *b* and *c* is 0.5 km. Originally the three sites were covered by rooted macrophytes but as eutrophication increased, together with human disturbance, *Zostera noltii* declined progressively, being currently restricted to site *a* (Fig. 1B).

Sampling was carried out almost each 2 months from February 1993 until December 2000 and monthly thereafter. From January 1997 to December 1998 no sampling was performed. On each sampling occasion, water temperature and salinity were recorded *in situ*. The water samples collected (approximately 250 ml) were stored, filtered through pre-combusted (3 h at 500 °C) GF/C filters (Whatman) in acid-washed polythene bottles at –18 °C until further analysis. Nitrate (NO₃-N) and nitrite (NO₂-N) were analysed according to standard methods described in Strickland and Parsons (1972) and ammonium (NH₄-N) and phosphate (PO₄-P) analysis followed the Limnologisk Metodik (1992) methodology. The phytoplankton chlorophyll *a* (Chl *a*) determinations were performed by filtering 0.5–1.0 L of water through Whatman GF/C glass-fibre filters followed by extraction according to Parsons et al.

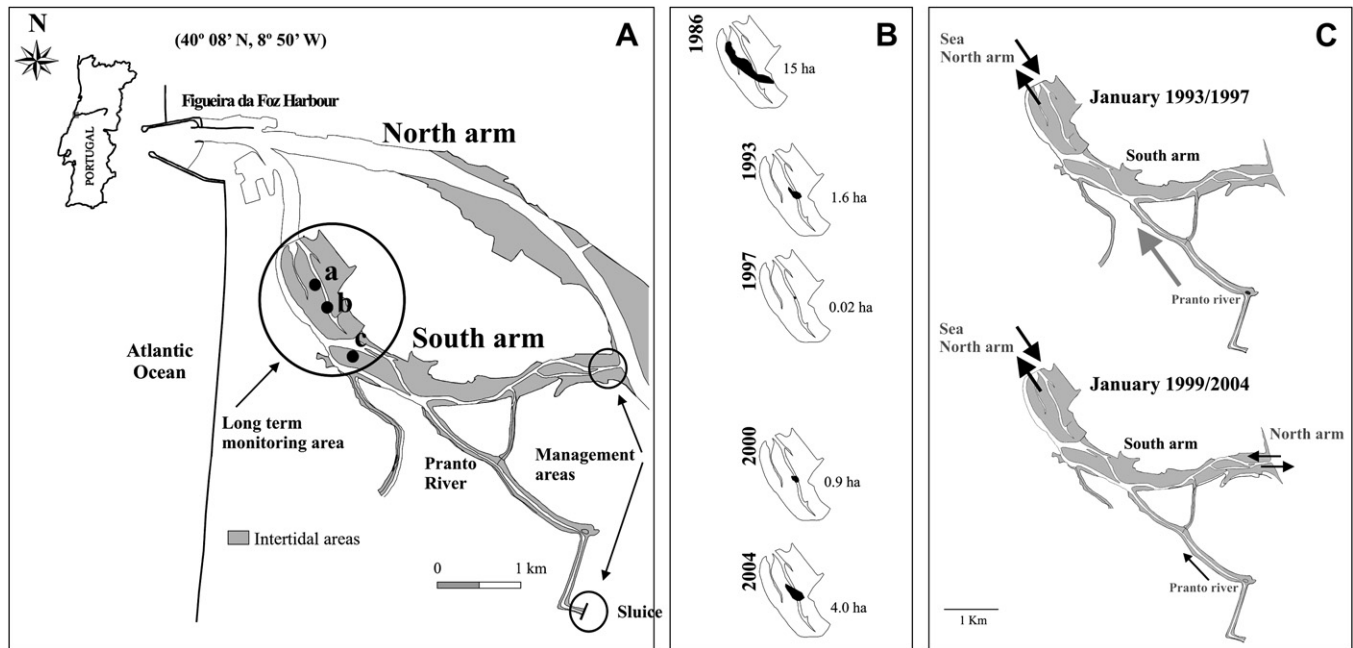


Fig. 1. (A): the Mondego estuary, with the location of the long-term monitoring area (*a*, *b* and *c* represent a gradient of eutrophication where *c* is the most eutrophicated area) and the areas where mitigation measures were implemented; (B): the evolution of the *Z. noltii* beds (ha) from 1986 to 2004, showing the drastic reduction from 1993 to 1997 and the recovery after 1998; (C): the main freshwater inputs before the management (1993–1997) and after (1999–2004). The black arrows represent saltwater and grey arrows represent freshwater flows into the system and their length indicates the intensity of the flow.

(1985). In the field and during transportation to the laboratory, samples were stored on ice and protected from light. Samples from the three monitoring areas (*a*, *b* and *c*) were collected and analysed separately. Due to the small distance between sites, data relating to the water column were put together and presented/related as mean values (\pm SE) for the South arm of the estuary.

Plant sampling for biomass assessment was performed haphazardly with a corer (minimum six cores, with 143 cm²

section) and biomass determined as ash free dry weight (AFDW) after oven drying at 60°C for 72 h and combustion at 450 °C for 8 h.

Macroalgae and seagrass for internal nutrient content were collected at site *a*, during low tide and transported in plastic bags to the laboratory where they were rinsed, dried for 48 h at 60 °C until constant weight and stored dry until analysis on internal N and P, in accordance to *Limnologisk Metodik* (1992). For phosphorus analysis, samples were combusted in

Table 1
Summary characterization of the South arm of the Mondego estuary

Characteristic	1993–1997 (Before management)	1999–2004 (After management)
Geographic location	40°08'N, 8°50'W	
System intertidal area (km ²)	1.75	
System subtidal area (km ²)	0.96	
System volume (10 ⁶ m ³)	5	
Mean depth (m)	2–4 high tide	
Tidal range (m)	0.35–3.3	
Mixing characteristics	Well-mixed with irregular river discharges	
Mean substratum composition	Silt, clay and sand	
Annual insolation of PAR (400–700 nm) (mol phot. m ⁻² y ⁻¹)	3200–32,000	
Salinity range	1.9–33.1	0.2–33.7
Mean water temperature range (°C)	8.0–23.7	8.1–22.1
Residence time	Moderate (weeks)	Short (days)
Current velocity	Low and dependent on the Pranto River sluice	Higher and not dependent on the Pranto River sluice
Turbidity	High	Lower
DIN (mean) (μmol L ⁻¹)	35.59	14.52
DIP (mean) (μmol L ⁻¹)	1.01	1.59
N/P (mean)	35.09	9.13

a muffle furnace at 500 °C for 3 h. All samples were homogenized prior to analysis.

2.3. Statistical analysis

The long-term data were analysed for significant changes before (1993–1997) and after the management (1999–2004) using the STATISTICA™ software (StatSoft Inc., 2001; version 6.0). Although the sets of data passed the Equal Variance Test, the Normality Test failed for all of the groups and for that reason the non-parametric Mann–Whitney Rank Sum Test was used to determine if the changes observed between both periods had statistical relevance.

3. Results

3.1. Nutrient concentration

Table 2 shows the changes in the nutrient concentrations present in the water column during the study period. The total dissolved inorganic nitrogen (TDIN) in the water column was lowered mostly due to the reduction of ammonium inputs, since the concentration of the oxidized forms (NO₃-N and NO₂-N) remained constant (n.s.: $T = 316.0$; $P = 0.602$) throughout the years (Table 2). The reduced form presented a mean value of 28.9 $\mu\text{mol L}^{-1}$ prior to 1998 which dropped to 7.76 $\mu\text{mol L}^{-1}$ afterwards (Table 2), with a statistically significant decrease (significant: $T = 44.0$; $P = 0.002$). Before the management actions, ammonium was the predominant form in the estuary about four times more abundant than the oxidized forms, but afterwards the ratio reduced:oxidized became 1:1.

The dissolved inorganic phosphorus (DIP) increased significantly (significant: $T = 147.0$; $P = 0.013$) from a mean value of 1.01 $\mu\text{mol L}^{-1}$ to 1.59 $\mu\text{mol L}^{-1}$ (Table 2).

3.2. Primary producers' biomass

The responses of the potentially opportunistic primary producers (chlorophyll *a* concentration and macroalgal biomass) for the two distinctive periods were different and are presented in Fig. 2. Phytoplankton data (concentration of chlorophyll *a*, Fig. 2A) are mean values for the three sites. To best describe the variations in biomass of macroalgae and seagrass, data were not presented as mean values. Instead, Ulvaceans biomass is described with data from site *c*, where they were continuously present throughout the study period (Fig. 2B).

Table 2

The water column nutrients concentrations ($\mu\text{mol L}^{-1}$) in the pre-management (1993–1997) and post-management (1999–2004) periods. SE is the standard error

	1993–1997				1999–2004			
	Min	Max	Mean	SE	Min	Max	Mean	SE
NO _x -N ($\mu\text{mol L}^{-1}$)	0.50	38.93	7.13	0.16	0.74	36.06	6.98	0.14
NH ₄ -N ($\mu\text{mol L}^{-1}$)	7.58	84.29	28.15	0.38	1.2	15.87	7.76	0.06
PO ₄ -P ($\mu\text{mol L}^{-1}$)	0.44	2.02	1.01	0.01	0.20	3.06	1.59	0.01

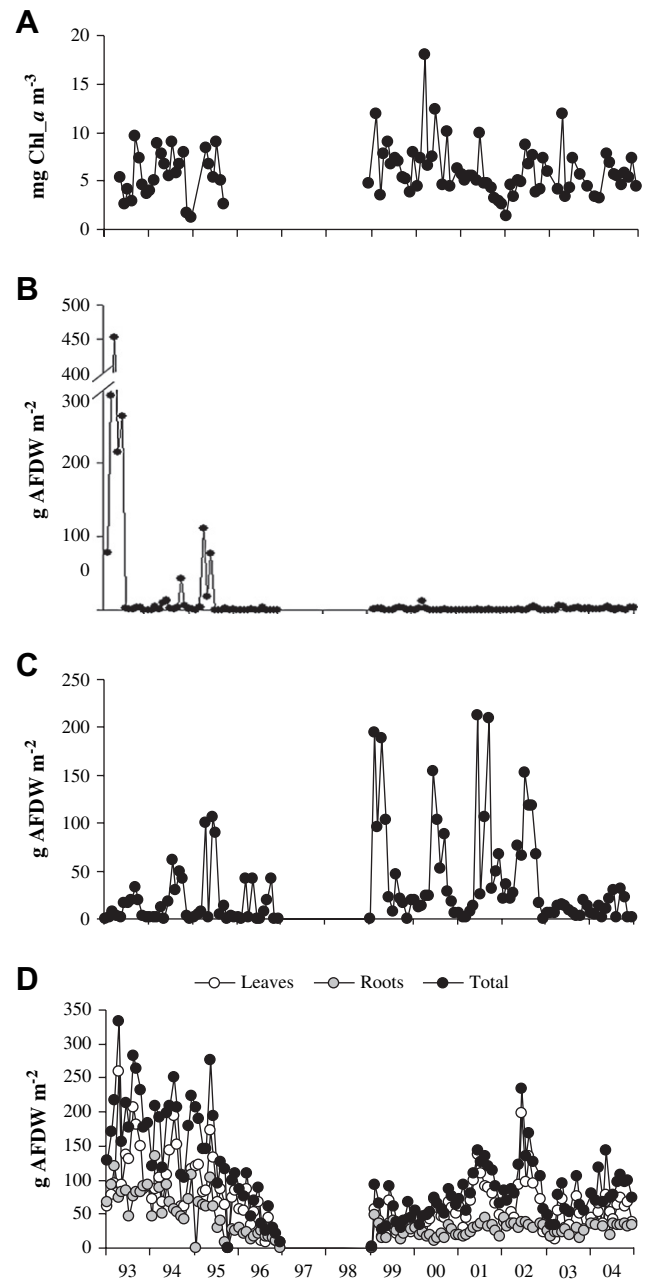


Fig. 2. The inter-annual variation of primary producers assemblages before (1993–1997) and after the management (1999–2004): (A): phytoplankton; (B): *Ulva* spp. biomass; (C): *Gracilaria gracilis* biomass; (D): *Z. noltii* biomass.

Gracilaria gracilis' presence, on the other hand, was intermittent at sites *b* and *c* but continuous at site *a* and for that reason data from this site were used (Fig. 2C). *Zostera noltii* was only present at site *a* (Fig. 2D).

Chlorophyll *a* concentrations (Fig. 2A) did not differ significantly ($T = 1120.5$, $P = 0.831$) between the two periods (Table 3).

With respect to macroalgae, the green species identified were *Ulva compressa* (L.) Greville and *Ulva intestinalis* (L.) Link (according to revision by Hayden et al., 2003) and the red macroalgae was *Gracilaria gracilis*. Ulvaceans presented

Table 3
Phytoplankton (Chl *a*) and macrophytes biomass and internal nutrient concentrations before (1993–1997) and after the management periods (1999–2004). SE is the standard error

		1993–1997				1999–2004			
		Min	Max	Mean	SE	Min	Max	Mean	SE
Chl <i>a</i> (mg m ⁻³)	Phytoplankton	1.20	20.97	6.80	0.91	1.43	18.05	6.12	0.04
Biomass (g m ⁻² AFDW)	<i>Ulva</i> spp.	0.00	452.23	34.66	13.06	0.00	12.63	1.52	0.24
	<i>Gracilaria gracilis</i>	0.00	105.9	17.63	2.50	0.00	213.27	41.14	6.40
	<i>Zostera noltii</i> roots	0.00	135.57	54.90	4.89	1.93	66.63	28.43	1.33
	<i>Zostera noltii</i> leaves	0.00	258.81	91.25	7.94	0.06	197.90	55.01	3.88
Tissue nitrogen (mg g ⁻¹ DW)	<i>Ulva</i> spp.	32.88	95.61	62.02	0.40	16.55	112.89	52.83	3.15
	<i>Gracilaria gracilis</i>	24.64	110.04	62.42	3.02	19.34	108.81	58.5	0.52
	<i>Zostera noltii</i> roots	11.56	45.56	23.92	2.85	3.37	47.42	19.14	1.91
	<i>Zostera noltii</i> leaves	19.47	64.08	37.10	3.72	6.05	45.37	29.54	1.45
Tissue phosphorus (mg g ⁻¹ DW)	<i>Ulva</i> spp.	1.19	5.54	2.18	7.51	0.29	3.87	2.04	0.12
	<i>Gracilaria gracilis</i>	1.23	2.88	2.08	0.05	0.05	6.23	2.25	0.03
	<i>Zostera noltii</i> roots	1.26	6.85	3.09	0.40	0.62	8.67	4.51	0.37
	<i>Zostera noltii</i> leaves	1.83	4.46	2.55	0.25	1.84	9.25	5.61	0.36

a very high biomass (Fig. 2B), with maximal densities of 452.23 g AFDW m⁻² and a mean value of 34.66 g AFDW m⁻² (Table 3), before the management actions were implemented. However, after 1998 green macroalgae showed a significant decrease in biomass ($T = 2754.5$, $P < 0.05$), with the mean value dropping to 1.52 g AFDW m⁻² (Table 3). On the other hand, *G. gracilis* biomass increased greatly ($T = 1984.5$, $P < 0.001$) (Fig. 2C). The highest value reported in the pre-management period was 105.9 g AFDW m⁻², with a mean value of 17.12 g AFDW m⁻² but afterwards it increased to 195.3 g AFDW m⁻² with a mean of 40.76 (Table 3). *Zostera noltii* meadows were dramatically reduced from an approximate area of 150,000 m² in 1986 to 200 m² in 1997 (Fig. 1B). Aboveground biomass suffered a drastic reduction between 1993 and 1997, but after 1999 it began to recover (Fig. 2D). However, the maximum biomass value for the post-management period (234.89 g AFDW m⁻²) is still inferior to the maximum value of 333.06 g AFDW m⁻² presented before the management. *Zostera noltii* aboveground and belowground biomass have shown significant differences ($T = 3021.0$, $P < 0.05$; $T = 3204.0$, $P < 0.05$, respectively) (Table 3) revealing an increasing tendency.

3.3. Seasonal growth pattern

Phytoplankton presented a seasonal variation throughout the study period, with chlorophyll increase in late winter/early spring, reaching a peak in mid summer and decreasing afterwards (Fig. 3A). The Ulvaceans biomass showed two distinct annual patterns. In 1993 and 1995, when large blooms of *Ulva* spp. occurred, growth began in late winter/early spring, with two peaks of biomass in mid spring and a subsequent decay in mid summer (Fig. 3B). In years with lower biomass, the pattern of growth of *Ulva* spp. often presented two peaks of biomass, one in mid spring and another in late summer/autumn (Fig. 3B). *Gracilaria gracilis* exhibited only one pattern of growth prevailing during the study period. Biomass production started in late winter, continuing throughout spring and

reaching a peak in summer (Fig. 3C). In autumn, biomass decreased but red macroalgae continued to be present all year round. *Zostera noltii* also exhibited a seasonal pattern of biomass production (Fig. 3D). Aboveground biomass increased mostly during the spring and summer, whereas the belowground biomass increased in autumn and winter.

3.4. Tissue nutrient concentrations

When considering the tissue N and P of green macroalgae throughout the study period, no statistically significant differences were found ($T = 221.5$, $P = 0.492$ for N; $T = 188.5$, $P = 0.788$ for P). The same situation was observed in *Gracilaria gracilis* ($T = 289.5$, $P = 0.844$ for N; $T = 244.0$, $P = 0.438$ for P). Macroalgae kept the internal nutrients' concentrations constant and with values within the same range (Table 3). Regarding *Zostera noltii*, the nitrogen content of the roots was not significantly different between the two periods ($T = 1.411$, $P = 0.166$), whereas nutrient content in leaves decreased significantly ($T = 2.310$, $P < 0.05$) from a mean value of 37.1 to 29.5 mg g⁻¹ (Table 3). The internal phosphorus content increased significantly both in roots ($T = -5.126$, $P < 0.001$) and leaves ($T = 2.343$, $P < 0.05$) (Fig. 4).

The analysis of the percentage of dry weight (% DW) in tissue nutrient concentrations allowed to determine if there were situations of nutrient limitation. The critical nutrient content for maximum growth is defined as the internal concentration that just limits maximal growth for plants (Hanisak, 1979). It was found that both macroalgae presented N and P values well above the critical tissue concentrations for maximum growth with an exception in 2000, when heavy rains washed away nutrients, causing P to drop below the critical level (Fig. 4A and B). Tissue nutrient content in *Zostera noltii* also indicated values above the critical level for both N and P (Fig. 4C and D). In 2003, the tissue N content of the roots was very close to the limit but it was still above the critical level (Fig. 4C).

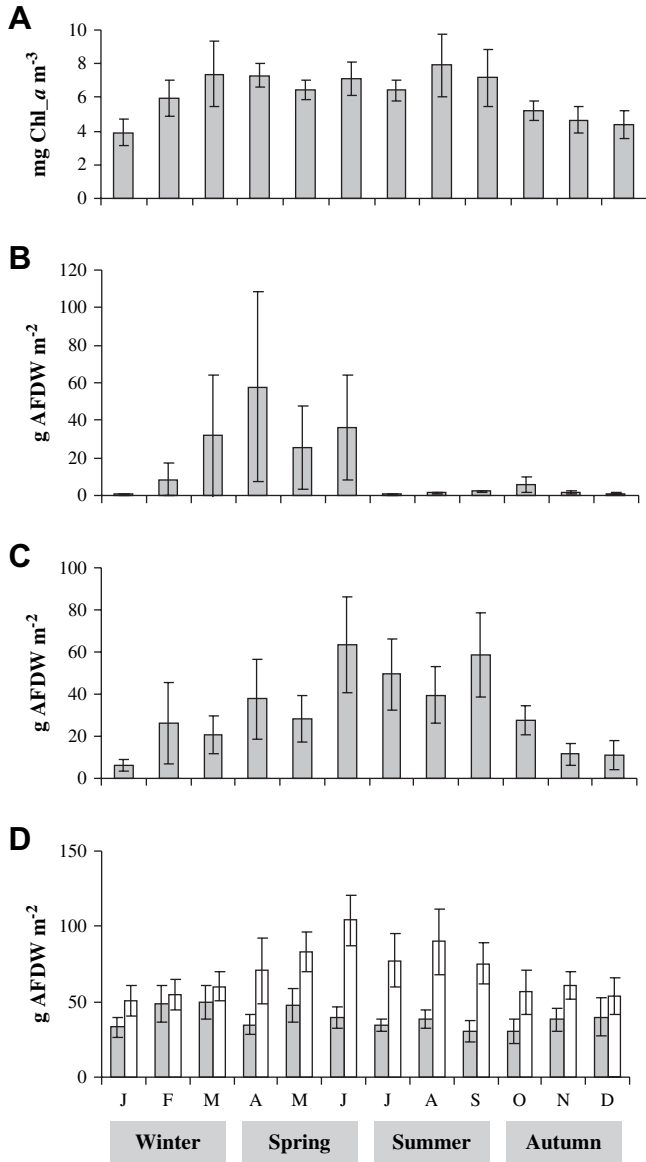


Fig. 3. The annual seasonal variation, considering the monthly means of: (A): phytoplankton; (B): *Ulva* spp.; (C): *Gracilaria gracilis*; (D): *Z. noltii* (grey bars representing roots biomass and the white bars represent leaves biomass), for the entire period of study (\pm SE, standard error).

4. Discussion

Several researchers have reported changes in community composition following N fertilization (Levine et al., 1998; Emery et al., 2001; Pennings et al., 2002). Some systems have shown a phytoplankton dominated-response (Taylor et al., 1995) while others presented macroalgal dominance (Lavery and McComb, 1991; Valiela et al., 1992). Shallow coastal estuaries with low nutrient availability in the water are more likely to be dominated by benthic algae and vascular plants due to the potential of these species to sequester nutrients from the sediments (Sand-Jensen and Borum, 1991; Havens et al., 2001). On the other hand, in systems with increased nutrient loading phytoplankton and/or macroalgae

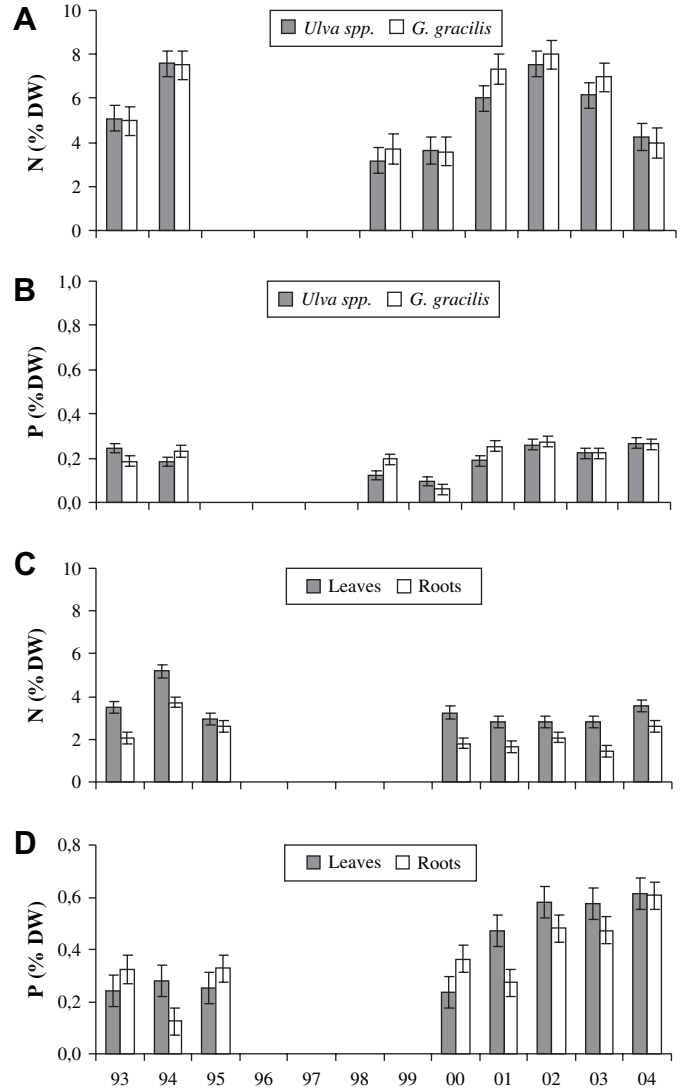


Fig. 4. The mean annual variations in the internal nutrients content as percentage of dry weight (\pm SE), in primary producer's assemblages, before and after 1998: nitrogen (A) and phosphorus (B) in macroalgae; nitrogen (C) and phosphorus (D) in *Z. noltii*. The grey bar represents the critical concentration value for maximum growth under which there is nutrient limitation (1.5% DW for nitrogen and 0.13% DW for phosphorus).

will dominate over vascular plants (Sand-Jensen and Borum, 1991. Valiela et al., 1997; Havens et al., 2001).

The Mondego estuary presented changes in the primary producers assemblages resulting from the restoration actions. Macroalgal assemblages showed different trends after 1998. Ulvaceans' biomass decreased while there was a significant increase in red macroalgae (*Gracilaria gracilis*), leading to a shift in macroalgal dominance. A similar change in the benthic community has been reported in Sacca di Goro, in north-eastern Italy, where the restriction of *Ulva rigida* resulted in the establishment of an almost monospecific algal cover of *G. gracilis* (Mistri et al., 2001). Simultaneously to the green macroalgal decrease in the Mondego's South arm, *Zostera noltii* slowly started to recover. Phytoplankton biomass, measured as concentration of chlorophyll *a*, did not present any

significant changes. These differences may be explained by a combination of changes in physicochemical and hydrological factors after the implementation of the mitigation measures, namely the improvement in water circulation which reduced the water residence time and the effective reduction in nitrogen loads (Lillebø et al., 2007) specifically ammonium.

Since chlorophyll *a* concentrations did not show any significant changes throughout the pre- and post-mitigation periods, it seems that phytoplankton biomass was not affected by the changes occurring in the system. Phytoplankton possess a lower compensation irradiance (Havens et al., 2001) and a comparatively higher surface area/volume relation that confers a higher affinity for nitrogen forms, with higher uptake rates than macroalgae for both ammonia and nitrate per unit of biomass (Hein et al., 1995; Collos, 1998). However, it is likely that there were changes in phytoplankton's composition but there is no data available to assess this.

Ulvaceans in the Mondego estuary have shown a decrease in biomass not accompanied by a decrease in internal nutrient content. In this system, the improvement of water circulation led to the increase in water flow and current velocity which in turn can lead to a higher export of floating macroalgae to the ocean (Flindt et al., 2004).

Green and red macroalgae have high affinity for ammonium and nitrate at both low and high concentrations (Fujita, 1985; Pedersen and Borum, 1997; Runcie et al., 2003) and are able to take up both forms simultaneously when they are present in the water column, but with different uptake rates (Kautsky, 1982; Pedersen and Borum, 1997; Glenn et al., 1999; Jones et al., 2001; Cohen and Fong, 2004). In *Ulva* spp. the uptake rate of the NO_3^- is about one-fourth of the uptake rates found for NH_4^+ (Runcie et al., 2003). This difference may be due to the nitrate's negative charge, which makes the uptake energy-dependent and thus slower (Runcie et al., 2003). After the uptake, nitrate must be converted into ammonium in a rate-limiting step catalyzed by nitrate reductase (NR) before being accumulated in vacuoles (Viaroli et al., 1996; Cohen and Fong, 2004; Lartigue and Sherman, 2005). The level of NR in *Ulva intestinalis* appears to be determined by the presence or absence of NO_3^- in the water column conferring macroalgae the ability to use a greater proportion of this nitrogen form. However, the uptake and assimilation of nitrate are energetically expensive, which could explain the preference for ammonium since it allows macroalgae to save energy (Cohen and Fong, 2004). It is possible that green macroalgae in the Mondego estuary, in order to maintain their nitrogen content, have taken up more nitrate to compensate the decrease in ammonium. Fong et al. (2004) hypothesized that *Ulva* spp. have evolved mechanisms to take advantage of high concentrations of nitrogen by prioritising allocation of available energy and carbon skeletons to nutrient uptake and assimilation of N. Turpin et al. (1988) described increased respiration rates and use of fixed carbon to synthesize amino acids following the addition of NO_3^- . This difference in uptake may have reduced the energy available and, as a result, maximum growth was not attained, even when the other environmental factors were favorable.

The changes in the nitrogen forms seem to have affected red macroalgae differently. Red macroalgae are especially efficient at taking up nutrients rapidly and, unlike Ulvaceans, have mechanisms to store large reserves of nitrogen (Jones et al., 2001; Menéndez et al., 2001; Hernández et al., 2006) in the red pigment phycoerythrin which in conditions of nutrient deficiency is quickly mobilized and used to sustain growth (Nagler et al., 2003; Hernández et al., 2006). This ability can constitute a competitive advantage over green macroalgae during periods of low nitrogen availability (Comín et al., 1995). Before 1998, the mean ammonium value in the water column was $28.15 \mu\text{mol L}^{-1}$, which means that ammonium uptake could be enough to maintain the internal nitrogen content above the critical tissue concentrations for maximum growth (1.5% DW for nitrogen according to Duarte, 1992). It seems possible that *Gracilaria gracilis* had lower biomass before the management due to shading by the green macroalgal mats lying above (Hernández et al., 1997). Also, during the growth seasons red macroalgae biomass increased after green macroalgae started to decrease and in years when there was an absence of blooms, *G. gracilis* biomass was higher, which seems to support this hypothesis. The fact that *G. gracilis* settles to the sediment may play an important role in nutrient acquisition and may be a competitive advantage since they can partially intercept nutrients released from the sediment that otherwise would enter the water column (Lavery and McComb, 1991; Menéndez and Comín, 2000). In this way, red macroalgae would have more ammonium available than green macroalgae after the management and would need to take up less oxidized forms, saving energy to maintain a high growth rate. Also, with the reduction of green biomass, light was more accessible to red macroalgae thus enhancing ammonium uptake and growth.

Seagrass species seem to be adapted to nitrogen-poor environments as they are able to maintain high production rates with relatively low nitrogen availability in the water column (Pedersen and Borum, 1992; Bocci et al., 1997; van Katwijk et al., 1997). This is possible due to an efficient uptake of nutrients from the water column and sediment pore water and to a mechanism of conservation, where older leaves act as nutrient sinks which afterwards are translocated to more actively growing and nutrient demanding tissues (Pedersen and Borum, 1992). Nutrients are taken up directly from the water column through leaves and from the sediment pore water through roots (Zimmerman et al., 1987; Hemminga et al., 1994; Bocci et al., 1997; Hemminga, 1998; Sfriso and Marcomini, 1999) but with differences in uptake (Rubio et al., 2005). Sediments are considered the primary nutrient source for seagrass roots, yet they may not have the capacity to support total nutrient requirements seeing as the uptake can be limited by diffusion (Touchette and Burkholder, 2000). In this way, leaf uptake represents an important contribution to nutrient supply. Leaves can take up both NH_4^+ and NO_3^- , whereas roots take up mostly NH_4^+ since it is the prevalent form in the sediments (Bocci et al., 1997). Nonetheless, aboveground tissues present a higher uptake affinity for NH_4^+ than roots and they seem to be more efficient in removing low levels of this reduced form (Pedersen

and Borum, 1992; Hemminga et al., 1994; Touchette and Burkholder, 2000). Considering that aboveground tissues are more sensible to light availability than belowground (Pèrez et al., 1994) and that they have an important function in nutrient absorption and in photosynthesis, seagrass would have suffered greatly from the presence of *Ulva* spp. mats. With the disappearance of the green blooms, light limitation was reversed and the inhibition on *Zostera noltii* eliminated. The decrease in internal N concentrations in the leaves after 1998 is possibly a combined result of ammonium reduction in the water column and a dilution effect due to increased biomass production.

Seagrass show a phosphate affinity in the same order of magnitude as for ammonium (Romero et al., 2006) and they tend to take up PO_4^{3-} mainly through the leaves, relying on root uptake only when it is negligible in the water column (Brix and Lyngby, 1985; Touchette and Burkholder, 2000). This would explain the increase in tissue P concentrations in both roots and leaves with the significant increase in DIP in the water column after the management actions were implemented.

5. Conclusion

The combined actions taken to reduce eutrophication symptoms in the South arm of the Mondego system have led to ecological changes within primary producer assemblages, namely: the beginning of *Zostera noltii* beds recovery, the increase of *Gracilaria gracilis* biomass, the absence of green macroalgal blooms, whilst phytoplankton biomass did not change. Therefore, since one action can cause different responses, it is important to try to understand what changes can be expected when management plans are implemented in the ecosystems.

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