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Land-derived materials are regulated by coastal and shelf environmental conditions before being transported to the open sea. It is of great concern to understand the processes and to establish the extent in which they modify terrestrial compound fluxes, such as nutrients, that end up in the oceans. At present, one of the topics that arouses the highest interest within environmental coastal studies is the direction and magnitude of inorganic nutrients and the exchange fluxes of organic matter between the water column and the associated tidal plains during the daily tidal cycle. These processes, together with the local hydrographic conditions, define the key role of this type of environment: its function as a nutrient and organic matter reservoir and/or as a source. A research programme directed to understand this mechanism within mesotidal estuaries was developed in the Bahía Blanca estuary, on the coast of Buenos Aires (Argentina). On a tidal time-scale basis, levels of DIN (nitrate + nitrite + ammonium), DIP, DISi, and organic matter were measured in the estuarine water column and tidal plain porewater, for two years. Results showed no significant variations during the tidal cycle, even though the temporal variation of these compounds was clearly identified. In addition, the biological production of the estuary was considered and taken into account, so as to understand the organic matter cycle within the system. Particular conditions of the environment (sediment characteristics, porewater chemical environment, hydrodynamics, anthropogenic sources, etc.) were also considered to help fully understand the results.

Keywords: mesotidal estuary; inorganic nutrients; organic matter; tidal cycle

1. Introduction

Physical and chemical dynamics, together with the ecology of shallow estuaries, are strongly influenced by the interactive mixing effect of both the runoff of freshwater from the land and the exchange of water with the adjacent open sea. The freshwater input influences estuarine hydrography by creating salinity gradients and stratification, assuring a large transport of silt, organic material and inorganic nutrients to the estuaries. The open marine areas impose large

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physical and chemical variations, which are forced on the estuarine ecosystem due to tide and wind generated water exchange. This pulse created by the boundary also ensures a large transport of organic material and nutrients subsidising primary and secondary production in the estuarine area [1]. Estuaries are usually characterised by an often well mixed, shallow water column, which results in tight coupling between benthic and pelagic processes. Then, organic matter is produced by a large variety of primary producers either connected to the sediment (i.e. microphytobenthos, macroalgae and rooted macrophytes), or in the pelagic, such as phytoplankton. Moreover, the occurrence of both efficient water column mixing as well as frequent resuspension events ensures fast vertical transport of organic and inorganic matter integrating the pelagic and benthic food webs, and making the biogeochemical processes function more lightly [2]. In estuaries with extensive intertidal flats, porewater exchange takes place between the sediment and the overlying water column for only a few hours, during high tide when the tidal flats are covered with water. After that, and for most of the tidal cycle, the tidal flats are exposed to the atmosphere [3]. During exposure, the redox zone can be modified as air floods the sediments, thereby changing the early diagenetic environment. Consequently, the understanding of the nutrient exchange that takes place in intertidal sediments is of great concern, in order to analyse and quantify the physical processes that govern water exchange between the periods of flooding by the tides [4].

Bahía Blanca estuary is located on the Atlantic coast of Argentina; it is a mesotidal estuary that includes very extensive tidal flats partially covered by halophytes (*Spartina densiflora* and *Sarcocornia perennis*), and they have been described as extremely productive [5].

The present study deals with the analysis of inorganic nutrients (nitrate, nitrite, ammonium, phosphate, silicate) and the seasonal distribution of organic matter as well as variation during the tidal cycle within the Bahía Blanca estuary. The budget of nutrient exchange between the tidal flats and the water column is also carefully considered.

2. Materials and methods

2.1. *Study area*

Bahía Blanca estuary (Figure 1) is formed by a series of NW-SE tidal channels separated by extensive intertidal flats, low marshes and islands. The northern area is geomorphologically dominated by the Principal Channel (Main Navigation Channel), while the southern area is dominated by Bahía Falsa and Bahía Verde, the largest channels within the estuary [6]. The Principal Channel covers a total length of 80 km and a width varying from about 3–4 km at the mouth (22 m depth) to 200 m at the head (3 m depth). The Principal Channel is partly closed by a modified ebb delta [7], as observed in other principal channels (bays) that flow towards the inner shelf.

The principal energy input to Bahía Blanca estuary comes from a standing semidiurnal tidal wave [6]. Strong NW and N winds dominate the typical weather pattern of the region, with a mean velocity of $24 \, km \, h^{-1}$ and gusts over $100 \, km \, h^{-1}$ [8]. Freshwater input is low on the northern coast from the Sauce Chico River (drainage area of $1600 \, km^2$) in the inner area, and from the Napostá Grande Stream (drainage area of $1237 \, km^2$) in the mid-zone of the estuary, both with an annual mean run-off of $1.72 \, m^3 \, s^{-1}$ (with a maximum flowrate of $18.32 \, m^3 \, s^{-1}$) and $1.05 \, m^3 \, s^{-1}$ (with a peak of $167.1 \, m^3 \, s^{-1}$), respectively [9]. The basin also includes small streams of discontinuous flow. The water column is vertically homogeneous all throughout the estuary, although it may be partially mixed in the inner zone depending on freshwater runoff conditions [6]. In addition, the inner zone is highly turbid as a result of the combined effect of winds and tide currents containing large amounts of suspended matter [6].

Dissolved oxygen (DO) values are usually close to saturation level values as a result of the high dynamics of the system, which stimulates both oxidation and re-mineralisation of organic matter
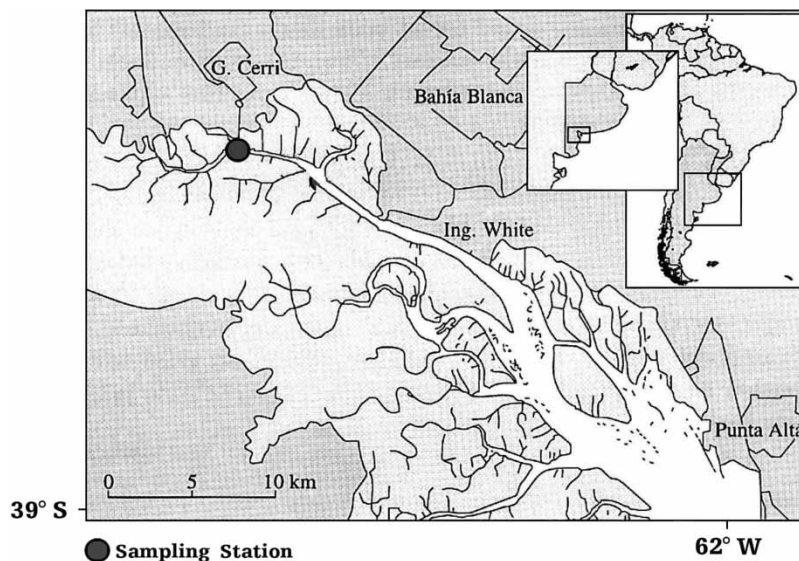
Figure 1. Location of the study area within Bahía Blanca estuary.

within the system. It is common to register supersaturating oxygen levels of up to 130% during the typical Winter/early Spring phytoplankton bloom within the estuary [5]. Nutrient concentrations are also high except during the bloom period, when they undergo a notorious decrease as a result of phytoplankton consumption [10,11].

Various ports, towns (with over 300,000 inhabitants) and industries are located at the northern boundaries of the estuary. Oil refineries and terminals, petrochemical industries, meat factories, leather plants, fish factories, textile plants, wool washing plants, silos and cereal mills discharge their processing residues into the streams or directly into the estuary. Significant volumes of raw sewage are discharged daily into the study area [12]. This estuary is extensively used by fishing boats, oil tankers and cargo vessels and requires regular dredging. In this sense, this coastal marine system receives contaminant inputs from municipal waste-waters, direct industrial discharges, harbour related operations and run-off waters that carry materials from land development areas as well as aerial fallout from atmospheric pollutants.

The study area has been located on the tidal flats close to Puerto Cuatreros, in the inner part of the estuary (Figure 1), considering that it has been recognised as representative of the upper part of the estuary, which is characterised by extremely turbid waters and high nutrient concentrations, as well as high phytoplankton biomass [10]. This area is characterised by the predominance of fine grain sediment, with a large abundance of mud and clays [13]. The semi-diurnal tidal regime in this area of the estuary includes a mean tidal amplitude of ~ 3.5 m, with variable velocity profiles according with corresponding dominant winds and meteorological conditions [14].

2.2. *Sampling and analytical procedures*

Estuarine water and tidal flat porewater samples were obtained in Puerto Cuatreros area, from the inner part of Bahía Blanca estuary (Figure 1) on a monthly frequency during two years (August 2004–June 2006). Each sampling campaign included *in situ* measurement of the water column's physical-chemical parameters (temperature, salinity, turbidity, pH and dissolved oxygen), as well as the collection of sampling water and pore water (for nutrients, organic matter and photosynthetic pigments), including three independent replicate samples for both matrices every hour along a

tidal cycle. These samples have been obtained once each hour from the low tide to the high tide, in order to assess the effect of the tide on the parameters values. A Horiba U-10 multisensor device was applied on the field for measurements, while pre-washed water samplers were used to get the corresponding samples, with cleaned plastic bottles to store them in at $-20\,^\circ$C (after filtration through Whatman GF-C filters with 1.2 μm pore size) until their analysis in the laboratory [15]. The corresponding filters with the retained suspended particulate matter (SPM) were stored ($-20\,^\circ$C) to determine organic matter and pigments [15].

For ammonium determination, samples were collected within glass tubes with a hermetic stopper, containing one drop of H_2SO_4 as preservative [15] and were kept under cool conditions until laboratory analysis.

Nutrient determinations were carried out using an expanded five channel Technicon II Autoanalyzer, following internationally standardised methods for nitrates and ammonium [16], nitrites [17], phosphates [18], and silicates [19].

Particulate organic matter (POM) content within SPM was determined according to [20], while both chlorophyll *a* and phaeopigments were measured according to [15]. Both analyses were performed using a Beckman DU-2 UV-V spectrophotometer.

Mean values of the studied nutrients and POM were compared using Tukey's test [21]. Factorial analysis was performed to reduce the number of variables recorded and to detect structure in the relationships between physico-chemical parameters and account for the variation present in the data set matrix. Factors were calculated from the correlation matrix and treated by Varimax rotation in order to maximise the load of each variable on one factor. Factors were extracted by principal components. All statistical tests were performed using the Statistica software package (Version 7.1). Significance was set at $p < 0.05$.

3. Results

High values of inorganic nutrients have been recorded in both the water column and the porewater of the studied tidal flats within Bahía Blanca estuary (Table 1). In the case of nitrogen compounds, two different trends have been observed during the study period. (i) The oxidised species (i.e. nitrite and nitrate) appeared to be slightly higher in the water column than in the porewater; even though no significant differences have been recorded between them, the corresponding ranges of the recorded values appeared to be quite different (Table 1). (ii) Reduced species (ammonium) levels were significantly higher ($p < 0.01$) in the porewater than in the water column, with quite different values (Table 1).

Furthermore, the study of phosphate showed no significant differences ($p < 0.01$) between the concentration in the water column and in the porewater (Table 1). Nor have silicate levels been significantly different ($p < 0.01$) between the water column or porewater (Table 1).

Moreover, the concentrations of POM as determined in the water column of the studied area appear to be significantly lower ($p < 0.01$) than those from the corresponding pore water (Table 1).

Table 1. Inorganic nutrients and particulate organic matter concentrations on the studied tidal flats within Bahía Blanca estuary. Range of values (after 24 campaigns). Mean value $\pm$ standard deviation in brackets.

	n	Water column	n	Porewater
Nitrate (μmol $\cdot$ L^{-1})	192	0.6–156 (6.5 $\pm$ 9.2)	144	0.2–133 (3.3 $\pm$ 4.9)
Nitrite (μmol $\cdot$ L^{-1})	192	0.01–18 (2.6 $\pm$ 5.1)	144	0.03–12 (1.1 $\pm$ 1.3)
Ammonium (μmol $\cdot$ L^{-1})	192	1.38–430 (23.1 $\pm$ 45.3)	144	0.06–965 (63 $\pm$ 39)
Phosphate (μmol $\cdot$ L^{-1})	192	0.3–64 (2.2 $\pm$ 0.9)	144	1.1–71 (1.7 $\pm$ 1.3)
Silicate (μmol $\cdot$ L^{-1})	192	86–804 (139 $\pm$ 131)	144	44–608 (199 $\pm$ 136)
Particulate Organic Matter (mg C $\cdot$ m^{-3})	192	164–3320 (1115 $\pm$ 872)	144	1100–15525 (2790 $\pm$ 1843)

The studied system proved to be productive during most of the year, with chlorophyll a values up to $30\,\mu g\cdot L^{-1}$ during the bloom periods, and mean values of $7.2 \pm 3.6\,\mu g\cdot L^{-1}$ (Figure 2). It is also important to highlight that the analysed nutrients were usually present within the studied system, and were never totally depleted during the recording period (Figure 3).

The factorial analysis of the physico-chemical parameters revealed that 73% of the total variance could be explained by four factors (Figure 4). The first factor (F1) accounts for 34% of the variance including NO_2^-, NO_3^-, pH and DO, associated with the emersion-immersion process. This factor
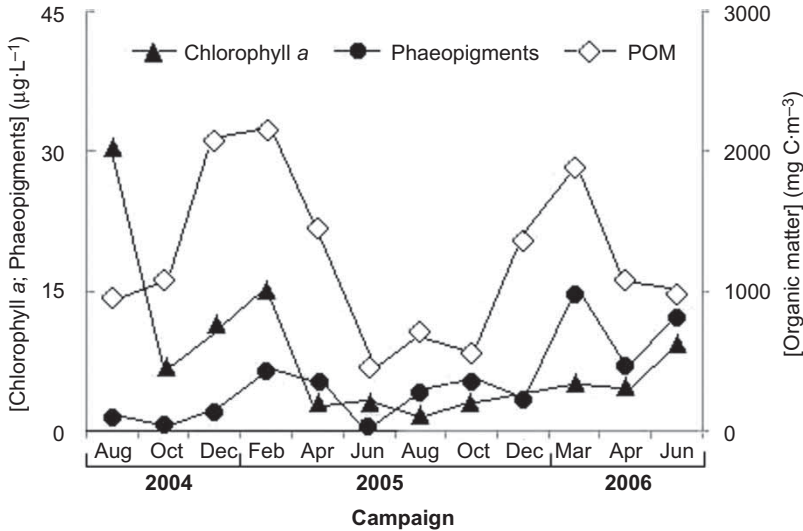
Figure 2. Distribution of chlorophyll a, phaeopigments and particulate organic matter in Bahía Blanca estuarine water along the study period.
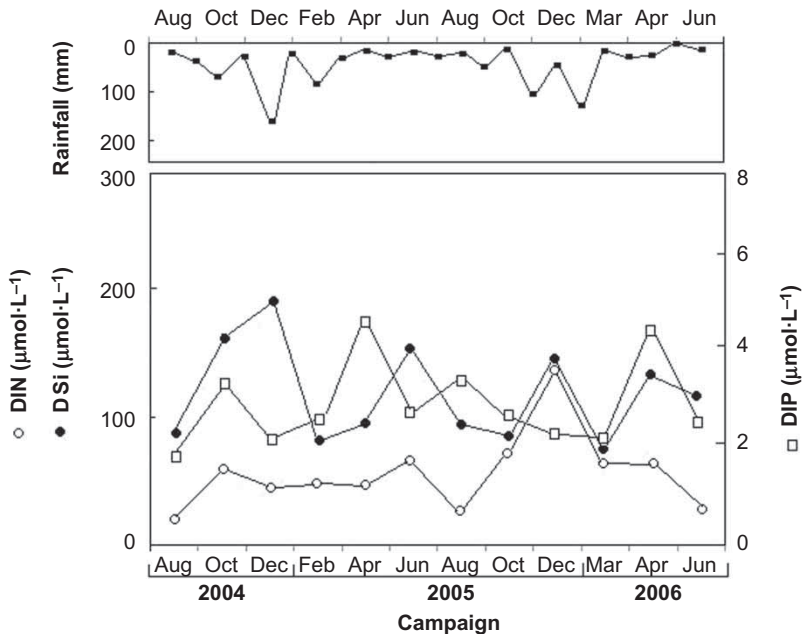

Figure 3. Distribution of dissolved inorganic nitrogen (DIN), phosphorous (DIP), silicon (DISi) and rainfall within the study area during the analysed period.
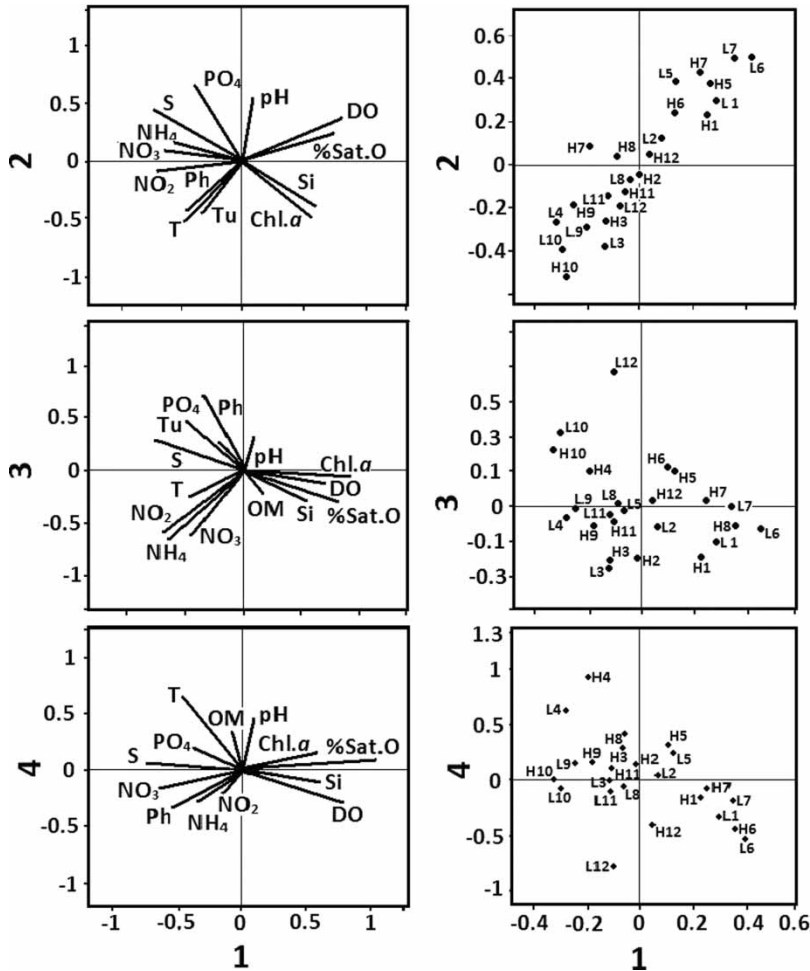
Figure 4. Correlation between variables and sampling sites within factors 1 to 4 (PCA analysis).

explains more than a quarter of the total variation, meaning that it is a dominant factor. Factor two (F2) is positively correlated with salinity (S), and negatively with temperature (T), explaining 18% of the variance, representing processes occurring during the high tide and low tide, respectively. The third factor, F3 (11% of total variance) presented a positive correlation with pH, turbidity and S, related to resuspension effects during both a rising tide and a descendant one. The fourth factor, F4 (10%), is represented by organic matter, S and pH, and negatively by DO, which could be related to the natural mineralisation processes.

3.1. *Variation during the tidal cycle*

Different trends have been identified in the analysis of nutrients and particulate organic matter level variation along the tidal cycle. The main results were:

(1) Ammonium concentrations seemed to increase in the water column from low tide (LT) to high tide (HT), reaching values from $\sim 30 \, \mu mol \, NH_4^+ \cdot L^{-1}$ to $\sim 65 \, \mu mol \, NH_4^+ \cdot L^{-1}$ (Figure 5); nevertheless there were no significant differences observed ($p < 0.01$) along the studied period. A similar trend was recorded for the porewater, but in this case concentrations varied
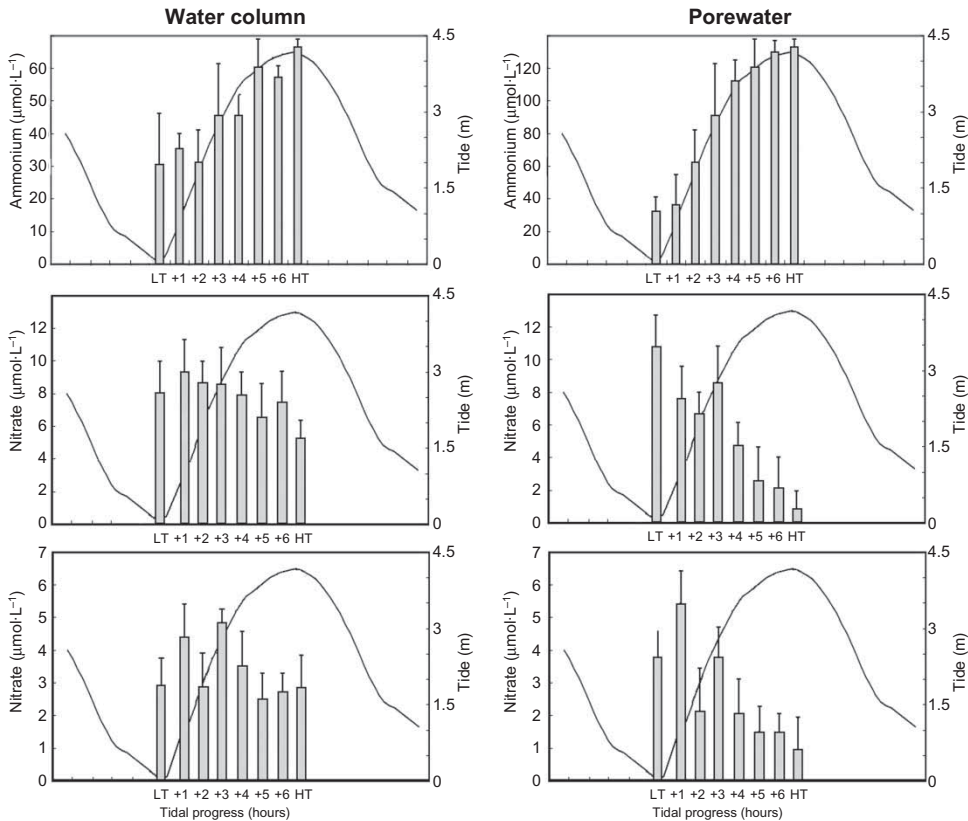
Figure 5. Variation of nitrogen nutrients concentrations along the tidal cycle in the water column and porewater within the studied area. LT, low tide; HT, high tide (bars represent mean values ± standard deviation after 24 sampling cruises).

from ~30 μmol $NH_4^+ \cdot L^{-1}$ to ~130 μmol $NH_4^+ \cdot L^{-1}$, denoting significant differences ($p < 0.01$) (Figure 5).

(2) Both nitrate and nitrite concentrations have presented no significant differences ($p < 0.01$) in the water column along the tidal cycle, though a slight decreasing trend could be observed within the corresponding results (Figure 5). On the contrary, a strong decreasing trend ($p < 0.05$) has been identified for both nutrients in the porewater, with extreme values, from ~11 μmol $NO_3^- \cdot L^{-1}$ (LT) to ~1 μmol $NO_3^- \cdot L^{-1}$ (HT), and from ~5 μmol $NO_2^- \cdot L^{-1}$ (LT) to ~1.5 μmol $NO_2^- \cdot L^{-1}$ (HT) (Figure 5).

(3) Both phosphate and silicate concentrations showed no significant differences ($p < 0.01$) during the tidal cycle, neither in the water column nor in the porewater (Figure 6). Nevertheless, a slight increasing trend has been identified for phosphate from LT to HT in the water column.

(4) POM levels increased significantly ($p < 0.01$) along the tidal cycle, varying from ~2000 mg C $\cdot$ m^{-3} (LT) to ~3400 mg C $\cdot$ m^{-3} (HT) in the water column, and from ~2700 mg C $\cdot$ m^{-3} (LT) up to 6700 mg C $\cdot$ m^{-3} (HT) in the porewater (Figure 6).

4. Discussion

The importance of quantifying the transport of nutritive substances to the coastal zone has been highlighted by the International Geosphere-Biosphere Programme (IGBP), through its Land-Ocean Interactions in the Coastal Zone (IGBP-LOICZ) [22], whose ultimate aim is to
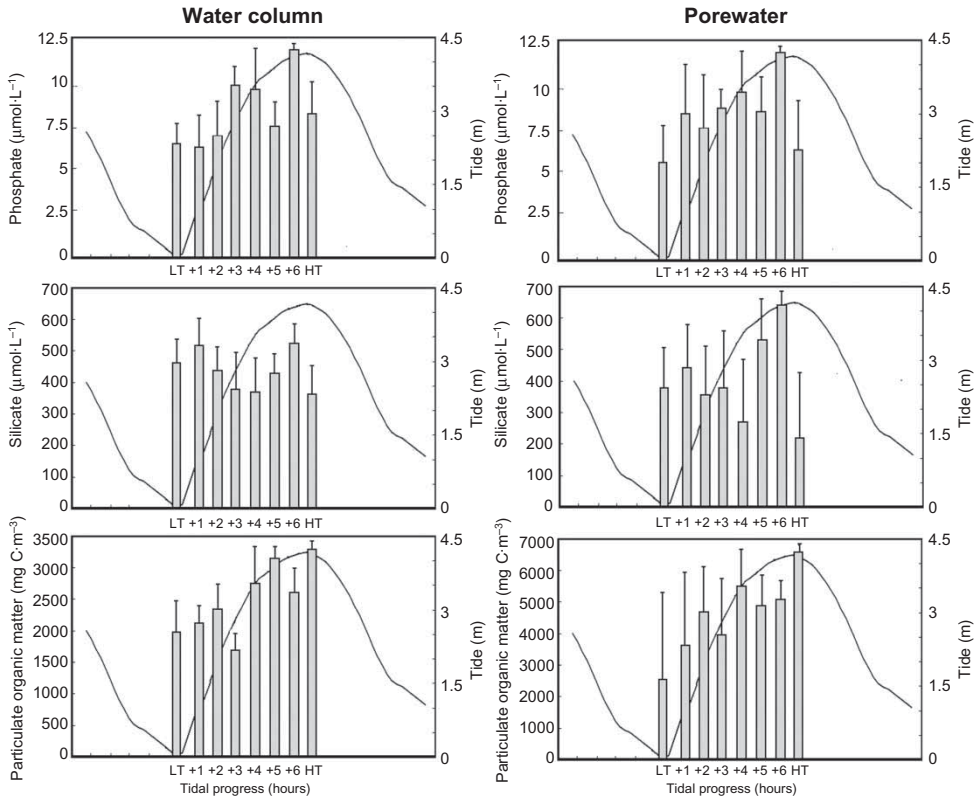
Figure 6.   Variation of phosphate, silicate and particulate organic matter concentrations along the tidal cycle in the water column and porewater within the studied area. LT, low tide; HT, high tide (bars represent mean values ± standard deviation after 24 sampling cruises).

develop a global inventory of nutrient budgets and to quantitatively understand the biophysical processes that regulate the Earth's surface and capacity to support life.

During the present study, the mainly considered inorganic nutrients were always present within the water column of the estuary, and none of them (DIN, DIP and DISi) was ever fully depleted (Figure 3). This was a remarkable outcome of the study considering that this type of process constitutes a strong support to primary production within the system, in agreement with previous reports within the area [5,10]. These nutrients could have different sources that must be thoroughly considered in order to understand their budget within the system.

Nutrient input from the Bahía Blanca estuary catchment should also be mentioned, considering it covers an extended surface ($\sim$7900 km^2) that is mainly used for agriculture and stockbreeding activities [9]. The runoff of this large area discharges great amounts of nutrients through the main freshwater courses within places such as the Sauce Chico river just in the inner part of the estuary, and the Naposta Grande stream towards the middle of it [23]; this relationship has also been observed in the present study, considering that the most intense rainfalls have quickly impacted on nutrient concentrations (Figure 3), which fully agrees with previous reports for other environments [24,25].

Another significant source of nutrients is the remineralisation process which occurs in the sediments of the estuary [26]. Bahía Blanca estuary has been described as an organic matter enriched system [11]; nevertheless, even though POM levels are high within the system (Figure 2), the corresponding historical mean level has not significantly increased [5], indicating that degradation

mechanisms are functioning in an adequate way. The efficient benthic microbial remineralisation of OM has been indicated as a significant tool for inorganic nutrient re-generation within coastal or estuarine sediments [27,28]. In addition, nutrient input through urban discharges within the inner area of Bahía Blanca estuary has opportunely been reported [29], including Bahía Blanca city and Pto. Ing. White sewage effluent systems which are discharged there after a preliminary treatment (only solid-liquid separation) [26]; this anthropic source was opportunely quantified [30], proving to be another significant source of nutrients and organic matter for the system. The integration of these inputs provides an adequate scenario to develop high primary production within the system throughout most of the year, with the highest peaks during Winter-early Spring, followed by late summer [10,31], and reaching primary production (PP) values up to $\sim$300 mg C $\cdot$ m^{-3} $\cdot$ h^{-1} [5].

Inorganic nutrient concentration, as determined in the present study within Bahía Blanca estuary, has shown to be among the highest reported for coastal and/or estuarine environments, agreeing with values presented for the Satilla River estuary (Georgia, USA) [32], or for the Minho River estuary (Portugal) [33], among others. On the other hand, even though Popovich et al. [10] have considered phosphorus as the main potential limiting nutrient for the Winter diatom bloom within the Bahía Blanca estuary, there was no evidence of it in the study period, and none of the considered inorganic nutrients – including P – have fully been depleted during this study (Figure 3). Despite this, and applying what was suggested by Rocha et al. [34], the limitation by nutrients was, calculated for the present data set; Figure 7 shows that most of the dots seemed to be plotted on the areas indicating the predominance of N or P as limiters, but their values are not so critical as to certify this kind of deficiency within the analysed system.

Thus, on the basis of the so-called Redfield ratios [35], commonly inferred as the molar ratios of nutrients in organic matter or, more specifically, phytoplankton (C : N : P = 106 : 16 : 1), some insight can be acquired regarding the relationship between *in situ* remineralisation and biological production. However, it must be remembered that an apparent shortage of a particular nutrient inferred from Redfield ratios does not necessarily imply limitation, since the target nutrient may
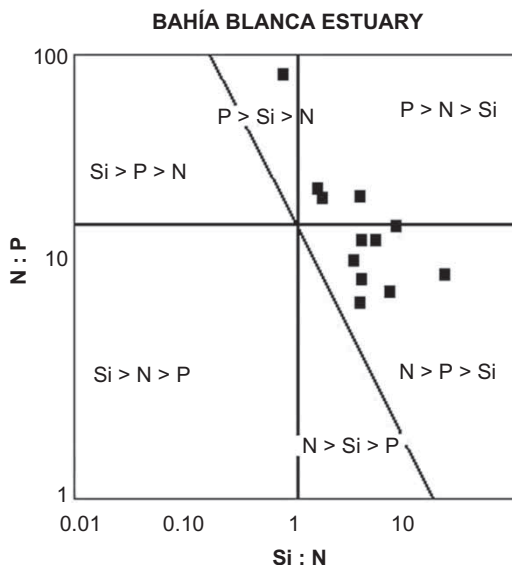

Figure 7. Diagram of nutrient limitations (in a Redfield ratio basis) calculated for the studied tidal flats within Bahía Blanca estuary (according to [34]).

be sufficient for phytoplankton metabolism. It is then convenient to regard such nutrient ratios as a tool for ascertaining potential nutrient limitation [28].

Moreover, trends identified in the analysis of nutrients and POM variation related to the tidal cycle within the tidal flats of the estuary indicated the occurrence of different processes that, as a whole, influenced the budget of these compounds within the Bahía Blanca estuary. At first, the changes recorded for the N compounds have showed a sustained variation pattern along the whole study period. The corresponding trends (increasing NH_4^+ in both WC and PW, no changes in NO_2^- and NO_3^- in WC, and a strong decrease in PW) led us to assume that significant chemical changes occurred during the tidal cycle (Figure 5). So, the stimulation of ammonium production rate in the interstitial water in proportion to the immersion time could indicate that the lack of oxygen due to immersion caused changes in the sediment redox environment and affected the nitrification and/or nitrate reduction rates [36]. The nitrate and nitrite pools in the porewater markedly decreased during immersion (up to 90% for nitrate and up to 76% for nitrite; Figure 5); however, this result could not be exclusively explained by molecular diffusion toward the water column, considering that the external forces caused by tidal currents and waves can account for the mixing of porewater with overlying water. In this sense, de Jonge and van Beusekom [37] speculated that the surface layer is more permeable as a result of hydrodynamic reworking. When sediments are exposed to air, the water table drops due to drainage and evaporation; on the contrary, during tidal flooding vertical infiltration of tidal water controls interstitial water levels [38]. This rhythmic emersion and immersion process can also mediate the below-ground transport of nutrients and organic matter, and in this way Howes and Goehringer [38] have reported the significant role of advective solute transport in the distribution of nutrients within salt marsh creek banks. During emersion, the penetration of oxygen into sediments may increase, producing changes in the redox environment; thus, oxygenation affects the rates and pathways of nutrients flow related to, e.g. aerobic nitrifiers and anaerobic denitrifiers [39]. The results of the present study fully agree with those by Usui et al. [40], who reported that porewater nitrate significantly decreased during the initial 3–4 hours after the onset of emersion. Moreover, Rocha and Cabral [36] have shown that approximately 80% of the nitrate pool flushed during immersion due to the mixing of porewater with overlying water, a fact that can result in drastic changes in the interstitial nutrient pool.

Neither phosphate nor silicate has shown significant variation during the tidal cycle (Figure 6). In this case, the low N:P relationship in the remineralisation zone may be a reflection of the faster kinetics of organic P remineralisation over organic N [28]. However, phosphate levels were almost continuously present within the system, bearing in mind that the high organic matter included in the sediments is fully oxidised there, releasing inorganic nutrients (phosphate among them) into the water column. Besides, silicates have been shown to be significantly contributed to through the catchment runoff, which has been recognised as silicate-enriched [26].

Briefly, both water column and porewater nutrient dynamics along the tidal cycle (at immersion and emersion) may be partially explained by these externally controlled physico-chemical interactions. Free convection due to the temperature gradients within sediments, and between sediments and flooding water, can also promote mixing of porewater with overlying water [36]. Many authors have suggested that macrofaunal reworking can cause much higher nutrient fluxes than molecular diffusion [41]. The macrofaunal abundance at Bahía Blanca estuary, and associated enhanced bioturbation, would also increase nutrient efflux from the sediments. All of the processes described above promote porewater and overlying water mixing.

Thus, and according to the present study, the mixing of lower-nutrient overlying water with nutrient-rich porewater results in porewater nutrient depletion by dilution. Therefore, the increase in porewater ammonium could be attributed to other mechanisms, including microbial remineralisation, stimulation due to bioturbation, sediment reworking, etc. Oxygenation during emersion facilitated the increase of oxidised compounds during this period.

5.   Conclusions

The main concluding comments obtained from the present study are the following:

- Bahía Blanca estuary has demonstrated itself to be a nutrient-enriched system, with high levels of these compounds along the whole year, and has dynamics strongly linked with the phytoplankton cycle within this environment.
- Ammonium was the main contributor to DIN, followed by nitrate and nitrite in both the water column and the porewater.
- Nutrient stocks have never been fully depleted during the study period.
- High levels of POM in the water column and extremely high levels in porewater have been determined, allowing the conclusion that the estuary is POM-enriched.
- Ammonium showed increasing concentrations from low to high tide in both WC and PW; levels were even higher in the latter.
- Nitrate and nitrite have presented slightly decreasing concentrations in WC and strongly decreasing concentrations in PW from LT to HT.
- Neither phosphate nor silicate showed significant differences in their concentrations, both in WC and PW, along the tidal cycle.
- POM was significantly lower in WC than in PW.
- The budget of nutrients and POM within the system has been related to diverse processes, and considering this it is not possible to explain comprehensively.

Finally, and keeping in mind the gaps as identified in the present study, future works will be necessary to fully understand the mechanisms that regulate the budget of nutrients and POM within this ecosystem.

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