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NUTRIENT BALANCE IN THE ECOSYSTEM OF THE NORTH WESTERN ADRIATIC SEA

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The effects of biological processes on dissolved inorganic nutrients, dissolved organic nitrogen (DON) and phosphorus (DOP) are considered in the north western Adriatic Sea. The budgets of these nutrients, which represent the sum of production and consumption processes in comparison to advection, are discussed with regard to dissolved inorganic nitrogen (¹⁵N labelled) uptake, which basically indicates the biological demand of this fraction of nitrogen by phytoplankton community.

The presented data show that, although important, the continental input of dissolved inorganic nitrogen (DIN), mainly nitrate, is utilised and recycled within the coastal marine environment (budget of $-15 \,\mu\text{mol-N} \cdot \text{dm}^{-3}$). In fact, during four cruises (June, 1996; February, 1997; June, 1997; February, 1998), phytoplankton production was mainly driven by regenerated nutrients ($f \le 0.4$). Regarding dissolved inorganic phosphorus (DIP), the negative budgets observed in most cases (down to $-0.4 \,\mu\text{mol-P} \cdot \text{dm}^{-3}$) confirm, above all, its scarce availability in this basin. Recycling processes rather than continental inputs regulate the availability of this nutrient. In addition, the comparison between DIN and DIP budgets indicates that, in this ecosystem, dissolved inorganic phosphorus is recycled faster than nitrogen through the living particulate and dissolved organic pools.

As a consequence of biological activities, a strong production of dissolved organic nitrogen (DON) can occur in summer (up to $+22 \,\mu$ mol-N dm⁻³) while DOP shows a more independent behaviour both with respect to its accumulation in the environment and to the observed nitrogen variations.

Keywords: Nutrient balance; Adriatic; nitrogen; phosphorus

1. INTRODUCTION

Knowledge of the biological pathways and of the budgets of inorganic and organic nutrients is fundamental for understanding the functioning of a marine ecosystem. This is particularly true in a basin like the north Adriatic Sea, where nutrient availability is strongly influenced by concomitant effects of relevant continental inputs, hydrological circulation and biological processes (Artegiani *et al.*, 1997; Degobbis and Gilmartin, 1990; Zoppini *et al.*, 1995). Nutrient fate, in principle, influences some of the major degenerative phenomena observed in the basin, like algal blooms, hypoxia and anoxia in the bottom layer and probably mucilage production (Degobbis *et al.*, 1995; Obernosterer and Herndl, 1995; Tommasino, 1996).

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This work aims to describe the effects of the most important biological phenomena acting on dissolved silicon, nitrogen and phosphorus in coastal waters, in order to understand their utilisation and fate in the ecosystem. We intend to discuss the balance of these nutrients as resulting from the sum of the biological processes of production and consumption (assimilation and regeneration) with respect to physical processes (continental inputs and mixing). Furthermore, the comparison between nutrient budgets and nitrogen uptake data allows us to recognise the role of different nutrient sources sustaining phytoplankton primary production and to obtain a better understanding of the functioning of the food web in different temporal situations. In fact, the prevalence of nitrate or of ammonium uptake indicates situations of prevailing new production, usually typical of an early bloom, or of regenerated production (Dugdale and Goering, 1967), characteristic of a mature bloom phase associated to important recycling processes.

The presented data were collected during four cruises carried out within PRISMA II "Biogeochemical Cycles" Research Project (June, 1996; February, 1997; June, 1997; February, 1998) in an area south of the Po River Delta (530 samples on the whole) which has been considered comprehensive both of advection processes, due to mixing between sea water and fresh water, and of biological processes, due to the presence of Po River, one of the greatest supplier of nutrients in the Adriatic basin (Fig. 1).

2. MATERIALS AND METHODS

At each station, temperature and salinity data were acquired during the downcast by means of a CTD probe, while sub-samples for the determination of inorganic nutrients and dissolved organic nitrogen (DON) and phosphorus (DOP) were collected during the upcasting by means of Niskin bottles mounted on a "rosette" sampler. Analyses of dissolved inorganic nitrogen (DIN = nitrates +nitrites +ammonium), dissolved inorganic phosphorus (DIP = reactive phosphorus) and reactive silicate (Si) were carried out directly on board, after filtration on Whatman GF/C filters, according to standard spectrophotometric methods (ALPKEM, 1992a; b; c; d). DON and DOP concentrations were determined as nitrates +nitrites and reactive phosphorus after photo-oxidation (UV +hydrogen peroxide) following the Walsh method (1989). In all cases when dissolved species had to be analysed, GF/C filters were adopted in place of GF/F for avoiding drawbacks due to slow filtrations and to an easy clogging of filters.

Nitrogen uptake rates by phytoplankton were determined with the use of 15 N labelled nitrate (Na¹⁵NO₃) and ammonium (15 NH₄Cl) according to the methods reported in the JGOFS Protocols (1994), and the procedures described by Owens (1988) and Owens and Rees (1989). Samples were collected, during upcast, at depths corresponding to 1%, 4%, 12%, 30%, and 100% of incident PAR penetration, then placed in on-deck incubators, maintained at sea-surface temperature and covered with perforated nickel screens to mimic the *in situ* irradiance conditions. After 24 h incubation, samples were filtered through pre-combusted Whatman GF/F filters, which retain mainly the phytoplanktonic component, and isotope enrichment of the particulate was determined by continuous flow nitrogen analyser-mass spectrometer (ANCA 20-20 MS, Europa Scientific). Nitrate and ammonium uptakes (QNO₃ and QNH₄) were used



FIGURE 1 Sampling stations of the four cruises of PRISMA II "biogeochemical cycles" research project (Δ = hydrological, nutrient and dissolved organic matter samplings; X = hydrological, nutrients and dissolved organic matter sampling and ¹⁵N uptake measurements). The salinities attributed to the end-members of the mixing process and used to calculate the nutrient budgets are also reported.

to calculate the f ratio (Eppley and Peterson, 1979) which represents an estimate of the importance of new vs regenerated production $(f = \text{QNO}_3/(\text{QNO}_3 + \text{QNH}_4))$.

3. RESULTS

The DIN, DIP, DON and DOP concentrations reflect the effects of many processes, including assimilation and regeneration in the water column, nutrient exchange with the sediments, advection processes and mixing between fresh water and sea water. In order to distinguish the influence of biological from physical processes, we have calculated the deviations (Δ -(i)) of field concentrations of each nutrient (i), with respect to the theoretical concentrations resulting on the basis of conservative mixing between fresh waters and marine waters (Fig. 1). These deviations are given by:

$$\Delta - (i) = C(i) - (C(i)_{riv} \cdot W_{riv}) - (C(i)_{sea} \cdot W_{sea})$$

where C(i) is the concentration of the nutrient (i) measured in each collected sample and $C(i)_{riv}$ and $C(i)_{sea}$ are the concentrations of the nutrient (i) in the two *end members*: the Po River water (ARPA Regione Emilia Romagna, Sede Provinciale of Ferrara, *pers. comm.*) and the open sea. The fresh water and sea water fractions in the sample (W_{riv} and W_{sea}) were calculated by assigning a salinity equal to zero to the Po River waters and by assigning a fixed value in each cruise (from 36.8 to 37.8) for the offshore sea water salinity. The sea water *end member* (C(i)_{sea} and W_{sea}) were evaluated as average of the data of the most offshore stations, at depths not influenced by continental inputs and where also nutrient assimilation and regeneration processes were balanced (oxygen saturation = 100%).

By assuming that physico-chemical processes connected with salinity changes (*i.e.* flocculation, adsorption/release equilibria on particulate) determine only a negligible influence in our system, a negative values of Δ -(i) means that the concentration of the nutrient (i), resulting from the mixing processes between fresh water and sea water, has been partially reduced by biological uptake acting there. When Δ -(i) > 0, regeneration processes prevail over uptake with a consequent increase in nutrient concentration. If Δ -(i) is close to zero, assimilation and regeneration are balanced so that biological activities do not affect nutrient concentration which can, thus, be considered driven only by mixing processes. In this case we will regard the nutrient behaviour as "*conservative*" or "*stationary*", *i.e.*, it is neither accumulated nor consumed. The scheme in Figure 2 shows the conceptual ecological significance of the calculated Δ -(i) values in comparison with ¹⁵N uptake data.

Figures 3, 4 and 6 show the relationships between the Δ -(i) values calculated for silicon and DIN, between DIN and DIP and between DON and DOP, respectively. In all these cases we will take into account two different layers distinguished on the prevalence of assimilation processes (*i.e.* negative Δ -(i) values) of reactive silicate, DIN and DIP, that normally occur in the upper layer (UL), or regeneration processes (*i.e.* positive Δ -(i) values) of these nutrients, usually associated to the bottom layer (BL). The ratios of assimilation and regeneration of inorganic nutrients are also evidenced (Figs. 3–4 and Table I), in both layers, by use of linear regression. They are determined as slope (X-coefficients) of the regression lines which indicate a ratio of assimilation, if related to negative Δ -(i) values, and a ratio of regeneration, if related to positive Δ -(i) values.



FIGURE 2 Conceptual scheme of the processes acting on the pools of dissolved inorganic and organic nutrients and ecological significance of inorganic nitrogen uptake and Δ -(i) budgets.



FIGURE 3 Comparison between the budgets of silicate (Δ -(Si), µmol-Si dm⁻³) and dissolved inorganic nitrogen (Δ -(DIN), µmol-N·dm⁻³) during the four PRISMA II "biogeochemical cycles" cruises. Plots show also the regression lines (solid lines) used to calculate Δ -(Si)/ Δ -(DIN) ratios (X-coefficients) of assimilation and regeneration and relative coefficients of determination (r^2).

3.1 Comparison between Silicon and DIN

Figure 3 compares the Δ -(i) values of silicon and DIN during the four cruises. Significantly negative budgets of these nutrients in the UL occur in summer (respectively down to -7μ mol-Si · dm⁻³ and down to -20μ mol-N · dm⁻³ in June, 1996 and 1997) and in winter (down to -20μ mol-Si · dm⁻³ in both February, 1997 and 1998) indicating an active consumption by a diatom rich phytoplankton community during all four cruises. On the contrary, BL show in summer positive values of Δ -(Si) (to $+20 \mu$ mol-Si · dm⁻³) and Δ -(DIN) (to $+15 \mu$ mol-N · dm⁻³) indicate a stronger remineralisation than in winter (up to+4 μ mol-Si · dm⁻³ and +4 μ mol-N · dm⁻³ respectively).



FIGURE 4 Comparison between the budgets of dissolved inorganic nitrogen (Δ -(DIN), µmol-N·dm⁻³) and dissolved inorganic phosphorus (Δ -(DIP), µmol-P·dm⁻³) during the four PRISMA II "biogeochemical cycles" cruises. Plots show also the regression lines (solid lines).

The coupling between consumption and recycling of silicon and nitrogen can be evaluated by the comparison of Δ -(Si)/ Δ -(DIN) ratios in upper and bottom layers. In both winters, this ratio is equal to 1.4 (Fig. 3b and Fig. 3d) in the entire water column, indicating a homogenous behaviour towards these two processes. On the contrary, a decrease of Δ -(Si)/ Δ -(DIN) ratio in the UL (0.5 and 1 in June, 1996 and 1997 respectively) and its increase in the BL (1.5 and 3) are observed during summer. Therefore the excess of assimilation of silicon in the UL and of remineralisation in the BL reveals the progressive uncoupling, passing from winter to summer, between silicon and nitrogen assimilation/regeneration process.

TABLE I Assimilation ratios of DIN (Δ -(DIN)/ Δ -(DIP)) and nitrate (Δ -(NO₃⁻/ Δ -(DIP)) compared to DIP (X-coefficients of the regression lines) in the UL, and relative coefficients of determination (r^2)

Cruise	Layer metres	Δ -(DIN)/ Δ -(DIP)		$\Delta - (NO_3^-)/\Delta - (DIP)$	
		ratio	r^2	ratio	r^2
Jun. 96	0–14	83	0.93	82	0.92
Feb. 97	0-15	42	0.81	44	0.82
Jun. 97	0-11	33	0.78	32	0.79
Feb. 98	0–19	30	0.95	29	0.95

3.2 Comparison Between DIN and DIP

The behaviour of Δ -(DIN) and Δ -(DIP) are shown in Figure 4. As already pointed out for silicon and DIN, the UL shows strong assimilation processes which determine negative budgets of DIP (down to $-0.4 \,\mu \text{mol-P} \cdot \text{dm}^{-3}$) in all periods. On the contrary, Δ -(DIP) in the BL shows always positive values (+0.1 $\mu \text{mol-P} \cdot \text{dm}^{-3}$ as maximum) and it is not characterised by seasonal variability as DIN and silicon.

In order to understand the role of the different fractions of nitrogen in the dissolved inorganic pool, it is useful to consider also the relationships between Δ -(DIP) and Δ -(NO₃⁻), Δ -(NO₂⁻) and Δ -(NH₄⁺) other than the Δ -(DIN) vs. Δ -(DIP) trend. In the UL, the negative Δ -(NO₃⁻) budget (down to -15μ mol-N · dm⁻³) matched against the Δ -(NO₂⁻) and Δ -(NH₄⁺) values, which are close to zero (from 1 to+1 μ mol-N · dm⁻³), indicates a net nitrate consumption and a conservative or stationary behaviour of nitrite and ammonium. This is also confirmed by the quasi-coincident values of Δ -(DIN)/ Δ -(DIP) and Δ -(NO₃⁻)/ Δ -(DIP) ratios shown in Table I.

Concerning the BL, the Δ -(DIN) budgets range from +2 to +15 µmol-N · dm⁻³. On the whole, regenerated inorganic nitrogen is mainly constitutes by nitrite and ammonium rather than nitrate: Δ -(NO₂⁻)+ Δ -(NH₄⁻) represents, on the average, the 75% of mineralised Δ -(DIN).

In this layer, Δ -(DIN)/ Δ -(DIP) ratios range from 26 to 58 thus indicating that the ecosystem processes many more moles of DIN for every mole of DIP. This can occur only within the hypothesis of compensation between the greater quantity of processed nitrogen and a higher turnover of phosphorus.

Focusing in particular on the capacity of the phytoplankton community to utilise the load of inorganic nutrients present in this system (white arrow in Fig. 2), inorganic nitrogen availability has been compared to phytoplankton nitrogen assimilation. Figure 5a and Figure 5b present the availability of nitrate and ammonium and their assimilation in the euphotic layer, which, as a matter of fact, corresponds to the UL previously defined.

Figure 5a clearly shows that June, 1996 and June, 1997 present very different nitrate + ammonium availability (average integrated concentrations equal to $0.7 \,\mu$ mol-N \cdot dm⁻³ in June, 1996 and equal to $3.8 \,\mu$ mol-N \cdot dm⁻³ in June, 1997) contrary to what occurs in February, 1997 and 1998 when concentrations were similar (equal to $3.8 \,\mu$ mol-N \cdot dm⁻³ in February, 1997 and equal to $3.3 \,\mu$ mol-N \cdot dm⁻³ in February, 1998) with a prevalence of nitrate (> $3 \,\mu$ mol-N \cdot dm⁻³) compared to ammonium (< $0.14 \,\mu$ mol-N \cdot dm⁻³). The varying nutrient availability does not seem to influence phytoplankton nitrogen uptake, as shown in Figure 5b and also revealed by the Δ -

(DIN) values (Fig. 4), which show similar DIN consumption in all periods. In fact, in June, 1996 and 1997, although DIN availability varies considerably (Fig. 5a), nitrate and ammonium uptakes remain almost constant (nitrate + ammonium uptakes $e = 0.43 \mu \text{mol-N} \cdot \text{dm}^{-3} \cdot \text{d}^{-1}$ in 1996 and $= 0.47 \mu \text{mol-N} \cdot \text{dm}^{-3} \cdot \text{d}^{-1}$ in 1997) with ammonium (Fig. 5b) as preferred substrate ($f \le 0.3$).

These results suggest that, in summer, the obtained values may represent the saturation limit for phytoplankton nitrogen uptake, notwithstanding the nutrient availability in the system. On the contrary, in February, 1997 and 1998, and in spite of a similar excess nitrogen availability with respect to phytoplankton demand, nitrogen uptake is significantly different both in the amount of nutrient assimilated (total nitrogen uptake in 1997 equal to $0.10 \,\mu\text{mol-N} \cdot \text{dm}^{-3} \cdot \text{d}^{-1}$ and $0.91 \,\mu\text{mol-N} \cdot \text{dm}^{-3} \cdot \text{d}^{-1}$ in 1998) and in the ratio between nitrate and ammonium uptake (f = 0.4 in 1997 and f = 0.7 in 1998).

On the basis of these considerations, therefore, only in February, 1998 the large availability of inorganic nitrogen seems to have been efficiently utilised by the phytoplankton community, being instead limited in summer.

3.3 Comparison between DON and DOP

In order to study the fate of nitrogen and phosphorus processed by the biological compartment, the budgets of DON and DOP have been taken into consideration (Fig. 6). These pools often constitute, in the North Adriatic basin, the most abundant fractions of total dissolved nitrogen and phosphorus in the water column (Lipizer *et al.*, 1997). However, DON and DOP fractions show independent behaviours when compared to DIN and DIP, which usually present covariant consumption and regeneration in both upper and bottom layers. This characteristic determines the scattered distribution of Δ -(DON) *vs* Δ -(DOP) values (Fig. 6) and it suggests different velocities of release/removal of DON and DOP in the ecosystem.

Considering their average values, Figure 6 presents, on one hand, the strong accumulation of DON in June, 1996 (Δ -(DON) = +6 µmol-N · dm⁻³) compared to the other periods (from -1 up to +1 µmol-N · dm⁻³) On the other, Δ -(DOP) average values (from -0.01 up to +0.02 µmol-P · dm⁻³) do not show any significant trends of accumulation and consumption, further sustaining the hypothesis of a phosphorus-limited system.

4. DISCUSSION

The calculation of Δ -(i) nutrient budgets and nitrogen uptake data highlight the prevailing role of biota, with respect to advection processes, in controlling silicon, nitrogen and phosphorus availability also in coastal waters directly affected by the Po River plume.

Concerning silicate, it is always strongly assimilated in the UL by a phytoplankton community rich in diatoms, thus giving negative values of Δ -(Si), down to $-20 \,\mu$ mol-Si \cdot dm⁻³, while it is strongly regenerated in the BL (up to $+20 \,\mu$ mol-Si \cdot dm⁻³) only in summer. The Δ -(Si)/ Δ -(DIN) ratio is equal to 1.4 in the entire water column in winter



FIGURE 5 Availability (a) and uptake (b) of nitrate (QNO_3) and ammonium (QNH_4) and calculated *f*-ratio measured in the euphotic layer during four PRISMA II "biogeochemical cycles" cruises. Values are the average concentrations and absolute uptake rates of nitrate and ammonium integrated within the euphotic layer depth.

but it becomes different in upper and bottom layers in summer. This suggests that, during the first phase of the phytoplankton bloom, the homogeneous water column forces the coupling between assimilation and regeneration. In summer, when the water columns, become stratificated, the progressive uncoupling between assimilation, mainly occurring in the UL, and regeneration, mainly in charge of the BL, becomes evident.

The comparison of the budgets of nitrate, nitrite and ammonium with DIP and the nitrogen uptake in the euphotic layer suggests some considerations on the different behaviour of these fractions of dissolved inorganic nitrogen.

In the UL, the similar values of Δ -(DIN) and Δ -(NO₃), both down to -15μ mol-N · dm⁻³, indicates that the strong assimilation of DIN can be ascribed to nitrate only. In addition, nitrate assimilated in the UL in all periods (QNO₃ from 0.04 to 0.64 µmol-N · dm⁻³ · d⁻¹) is not quantitatively restored. After a phase of prevailing new production, the quasi-conservative budgets of nitrites and ammonium (from -1 to $+1 \mu$ mol-N · dm⁻³) and the presence of an important ammonium uptake (QNH4 from 0.06 to 0.37 µmol-N · dm⁻³ · d⁻¹) suggest that recycling processes in the water column become the major source of nitrogen for assimilation, as also confirmed by the low *f* ratios ($f \le 0.4$). The differences observed between the constantly high negative DIN budget in the UL (Δ -(DIN) = -15μ mol · dm⁻³) and the variable nitrogen uptake by phytoplankton (from 0.10 up to 0.91 µmol · dm⁻³ · d⁻¹ of nitrogen) suggest an increase in



FIGURE 6 Comparison between the budgets of dissolved organic nitrogen (Δ -(DON), µmol-N·dm⁻³) and dissolved organic phosphorus (Δ -(DOP), µmol-P·dm⁻³) during the four PRISMA II "biogeochemical cycles" cruises. Numbers in the plots indicate the average of Δ -(DON) and Δ -(DOP) values.

competition between different compartments of the food web with the progress of the bloom. Only in February, 1998, during an early bloom situation revealed by the high *f*-ratio (f=0.7), high primary production (Vadrucci *pers. comm.*) and still high nutrient availability, phytoplankton seemed to be the main responsible of nutrient assimilation.

Considering the BL, regeneration of dissolved inorganic nitrogen (to $+15 \,\mu$ mol-N \cdot dm⁻³ in summer) is the main cause of nitrite and ammonium enrichment, which can constitute up to 75% of the DIN. With regard to inorganic phosphorus, the prevalence of negative Δ -(DIP) in the entire water column (from -0.4 up to $+0.1 \,\mu$ mol-P \cdot dm⁻³) agrees with the hypothesis which depicts the North-Adriatic as a phosphorus deficient basin.

The higher Δ -(DIN)/ Δ -(DIP) ratios in both assimilation and remineralisation (from 26 to 83) compared to phytoplankton (N : P = 16 : 1, Redfield *et al.*, 1963) and bacteria (NP = 9 : 1, Goldman *et al.*, 1987) N/P composition ratios, suggest that faster recycling of phosphorus must account for the higher nitrogen assimilation within the time-scale

used to calculate the Δ -(i) budgets. In particular, if the efficiency of DIP recycling is evaluated on this basis, in June, 1996 this efficiency was two times greater than in June, 1997.

Finally, the independent behaviour of the DON and DOP and the highest accumulation of DON (Δ -(DON) to +22 µmol-N · dm⁻³ in June, 1996) not balanced by a similar DOP accumulation suggest a possible phosphorus limitation also for organic nitrogen demand.

5. CONCLUSIONS

These considerations allow us to conclude that the north western Adriatic Sea actively processes the large continental inputs mainly deriving from the Po River. Remineralisation processes are quite significant as revealed by the prevalence of regenerated compared to new phytoplankton production. This observation is also confirmed by the net consumption of new DIN inflowing in the system, basically consisting in nitrates, and the almost conservative budgets of recycled nutrients, nitrites and ammonium, which are continuously consumed and restored. The significance of recycling is evidenced by the comparison between the almost constant inorganic nitrogen consumption in the upper layer and the variable nitrogen uptake and low f ratios derived from direct measurements.

The prevalence of negative budgets of both inorganic and organic phosphorus implies the deficiency of this element in the system, while the high DIN/DIP ratios suggest the necessity of a more efficient recycling of phosphorus than nitrogen through the organic pools. The consequent uncoupling may determine a large accumulation of DON with respect to DOP.

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