Porewater Nutrient and Oxygen Profiles and Sediment-Water Interface Fluxes Under Extreme Organic Loading in Different Sedimentary Habitats in Sozopol Bay (SW Black Sea): A Laboratory Experiment

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Abstract Coastal benthic sediments play an important role in regulating water column nutrient concentrations and primary production via nutrient regeneration and exchanges at the sediment-water interface. This study aimed to characterize the porewater concentrations and diffusive benthic fluxes of NH_4^+, NO_3^-, PO_4^{-3}, and O_2 in some of the most common shallow sedimentary habitats (fine and coarse sands, seagrass beds, and unvegetated patches within the seagrass beds) along the Bulgarian coast, and their changes under organic loading, through a laboratory experiment. Ammonium was the dominant form of nitrogen in porewaters, and its concentration generally increased under organic loading in most sediment types. Nitrate concentrations were high in the overlying water, and decreased with depth within the sediments, becoming depleted at ~3 cm with the development of anoxic conditions. Phosphate concentrations were low, and tended to increase with depth by the end of the experiment in most sediment types and especially under organic loading. Nutrient fluxes were dominated by a release of NH_4^+ to the water column in all sediment types, and a parallel uptake of NO_3^- by the sediments; both fluxes increased under organic loading, possibly indicating stimulation of nitrate reduction within the sediments. The PO_4^{-3} fluxes were smaller, and the sediments mostly acted as a source for phosphorus under organic loading. O_2 was taken up from the overlying water in all treatments and sediment types, and this flux increased under organic loading, probably in relation to the decomposition of the organic matter and spontaneous chemical oxidation of sulphide ions, released during sulphate reduction within the sediments. The study contributes towards the understanding of nutrient cycling and the role of the benthic compartment in Black Sea coastal soft-bottom habitats.

Keywords Benthic fluxes · Sediment-water interface · Pore waters · Nutrient recycling · Coastal zone · Black Sea

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N. Dobrinkova and G. Gadzhev (eds.), *Environmental Protection and Disaster Risks*, Studies in Systems, Decision and Control 361,
https://doi.org/10.1007/978-3-030-70190-1_25

1 Introduction

Coastal ecosystems, located at the interface of land and sea, and home to a large and continuously growing proportion of the global human population [1], are subjected to various natural and anthropogenic pressures. Yet, these are some of the most diverse ecosystems, with a wide variety of habitats, and an important part of global biogeochemical cycles, acting as a buffer for the open ocean from anthropogenic nutrient inputs [2]. Eutrophication resulting from increased nutrient inputs to the coastal zone, and consequent deterioration in water quality, continue to be reported worldwide, despite legislative and management measures taken in recent years to reduce loads [3, 4].

Particulate nutrients deposited in coastal sediments may either recycle back into the water column or become retained or transformed in the seabed, depending on physicochemical and biological factors, and particularly the oxygen concentration in the bottom water and advective transport [5, 6]. The benthic compartment can thus act as either a source or a sink for nutrients, and can exert control on the nutrient levels in the overlying water column, especially in shallow environments. The release of nutrients can sometimes lead to secondary benthic-driven eutrophication, particularly via sediment resuspension or diffusion from the sediments to water columns depleted of nutrients from high levels of primary productivity in summer [6, 7].

Nutrient porewater profiles have been used as an indication for biogeochemical reactions taking place within the sediments, in order to characterize the role of the benthic compartment in biogeochemical cycles (e.g. [8–10]). Fluxes at sediment-water interface, linking the benthic with the pelagic compartment, also represent mineralization processes within the sediments and nutrient regeneration, and are a crucial factor affecting nutrient balance and primary productivity in the water column, and from there—coastal water quality [11]. While many benthic flux studies have focused on compact, non-permeable silts, sandy sediments, with their varying topography and sometimes deep pore water penetration, can also provide conditions for efficient remineralization of organic matter and nutrient exchange. Additionally, sands are some of the most common and widely distributed benthic substrate types, and therefore likely play a significant role in biogeochemical processes, acting as filters where organic matter inputs from the water column are quickly decomposed [12, 13].

Along the Bulgarian Black Sea coast, nutrient fluxes at the sediment-water interface have been studied primarily in silty sediments. Doncheva and Shtereva [14] registered the highest nutrient fluxes in front of Kamchia River, attributable to the riverine inputs. Doncheva [15] also found very high sediment-water fluxes in Varna Lake and Varna Bay, especially in summer, which were enough to support hyper-eutrophic conditions in the lake, and eutrophic—in the bay. However, there are few studies on these processes in the southern Black Sea, where Burgas Bay—the other large bay along the Bulgarian coast, with potentially high water residence times, and which acts as a source of nutrients to the neighbouring coastal waters—is located [16–19].

This study aims to quantify the porewater nutrient concentrations and the interfacial diffusive fluxes in different sedimentary habitat types in Sozopol Bay, and their changes under experimental organic loading, through a laboratory experiment simulating an extreme eutrophication event.

2 Materials and Methods

2.1 Study Area and Field Sampling

Sozopol Bay is a semi-enclosed bay located in the southern part of the Bulgarian Black Sea, within the larger Burgas Bay. The bay is part of the NATURA 2000 protected area BG0000146 "Plazh Gradina-Zlatna Ribka". The majority of the area is marine, and includes three seagrass meadows, different soft bottom biotopes, and reefs with macroalgae and black mussels. The area is strongly influenced by different anthropogenic pressures from the town of Sozopol, extensive tourism, and fisheries industries in the area.

Sampling was carried out during the summer of 2019. 3 replicate core samples (internal diameter 10.5 cm, sampling depth ~20 cm) were collected by Scuba divers from 4 different sedimentary habitats in Sozopol Bay, representing the most common substrates in the area (Fig. 1).

Station 1 has coarse sandy substrate (mean grain size 0.5–1 mm according to the Folk and Ward classification [20]; station 2—fine sand (mean grain size 0.125–0.25 mm), station 3—seagrass meadow (Zostera marina and Zostera noltei), with predominantly fine sandy substrate mixed with a little silt; and station 4—an adjacent unvegetated patch within the seagrass meadow ("no Zostera"), with similar substrate, occasionally mixed with mollusk shells and fragments. All stations are located at 4–4.5 m depth. The main characteristics of the sampling sites are presented in Table 1. One of the coarse sand samples was disturbed during handling and transportation, leaving only 2 for that habitat type.

2.2 Laboratory Experiment

In the laboratory, samples were placed in a gently aerated aquarium, and left to acclimate in near-natural conditions for 1 month. Distilled water was added weekly to maintain water level and salinity. After this period, one replicate per habitat type was used to measure sediment parameters (water content, porosity) and organic matter content. Nutrient ($N–NH_4$, $N–NO_3$, $P–PO_4$) and oxygen concentrations were measured in the water layer immediately overlying the sediment surface, and in the pore waters within the sediments in each of the remaining cores. Pore waters were sampled each cm to 10 cm depth, and oxygen was measured each cm until the

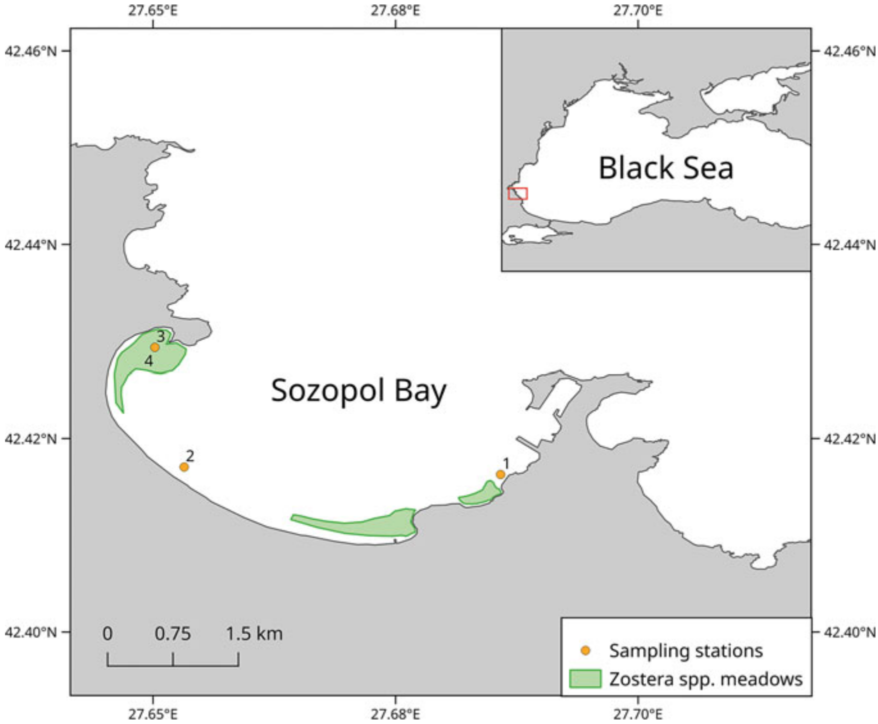

Fig. 1 Study area and sampling stations. 1—coarse sand, 2—fine sand, 3—seagrass meadow (Zostera), 4—unvegetated patch within seagrass meadow (no Zostera). Stations 3 and 4 are adjacent, located within several metres of each other, and so share the same coordinates

Table 1 Surface sediment characteristics (top 1 cm) at the sampling stations, with the average values for each sample shown in brackets. %TOM—% total organic matter content

Station	Habitat type	%TOM	Porosity ($mL\ cm^{-3}$)	Bulk density ($g\ cm^{-3}$)	Water content (%)
1	Coarse sand	1.38 (1.35)	0.34 (0.34)	1.09 (1.17)	23.73 (22.60)
2	Fine sand	1.28 (1.36)	0.33 (0.19)	1.13 (0.65)	22.33 (22.34)
3	Zostera	1.47 (1.45)	0.58 (0.41)	1.55 (1.13)	27.17 (26.66)
4	No Zostera	1.17 (1.33)	0.46 (0.37)	1.56 (1.24)	22.52 (22.91)

Bulk density is derived from dry sediment weight

detection of persistent anoxic conditions. The nutrient concentrations were measured according to [21]. The oxygen concentrations were measured with a microelectrode (MC100 Microcell O_2 m). Organic matter was determined as weight loss on ignition at 520 °C. One of the two replicates per habitat type was loaded with 6 g dried and finely ground green algae deposited on the sediment surface, or 0.069 g cm^{-2}—about double the normal surface organic matter content in a typical seagrass bed in the study area (0.03–0.04 g cm^{-2}) [22]. Effectively, this simulates organic loading from an extreme eutrophication event. The other replicate served as control. The sole remaining coarse sand sample was loaded with organic matter. The experiment continued for 39 days, after which the pore water nutrients and oxygen, and the sediment parameters in each sample were measured again according to the same protocol.

2.3 Flux Calculations

Diffusive fluxes at the sediment-water interface were calculated according to Fick's first law, corrected with the porosity and tortuosity for the particular sedimentary matrix, and expressed in mmol m^{-2} day^{-1} (nutrients) and mg m^{-2} day^{-1} (oxygen), with positive values representing uptake by the sediments, and negative—release to the water column:

$$F = -\frac{\phi D_0}{\theta^2}\frac{dC}{dx}t$$

ϕ—sediment porosity at the interface; calculated from the sediment samples D_0—diffusion coefficient of the solute in seawater without the presence of the sediment matrix, corrected for average temperature (25 °C for the start and 21 °C for the end of the experiment) and salinity (16.5) at the study site and during the experiment [23] (Table 2).

θ—sediment tortuosity (dimensionless), expressing the influence of the sediment matrix on the diffusion, and calculated based on the porosity as $\theta^2 = 1 - 2\ln(\phi)$. dC—difference in concentration of the solute between the pore water at a particular depth and in the seawater immediately overlying the sediment surface dx—distance (cm) which the ion has to migrate from that depth to the sediment surface t—time, as number of seconds in a day (86,400).

Table 2 Specific diffusion coefficients for the solutes in seawater at 21 °C (D21) and 25 °C (D25)

Solute	D21	D25
O_2	0.195	0.216
NH_4	0.176	0.191
NO_3	0.170	0.185
PO_4	0.054	0.060

3 Results

3.1 Porewater Nutrient and Oxygen Profiles

Porewater oxygen concentrations sharply decreased, from 7 to 8 mg L^{-1} in the water overlying the sediments, to 0 at the 1–2 cm in most sediment types; only the sediments from the seagrass bed had higher oxygen penetration depth (3–4 cm) (Fig. 2). By the end of the experiment, all sediment types had become anoxic below 2 cm depth in both control and treated conditions.

Ammonium was the main form of dissolved inorganic nitrogen in the pore waters. Its concentrations also increased with depth in the sediment as the latter became more reduced. Under organic loading, the porewater NH_4^+ concentrations increased, but the shape of the vertical profile did not change. No change was observed in the seagrass sediments (Fig. 2).

The concentrations of nitrates were relatively high in the overlying water—the highest of any of the measured nutrients; within the pore waters, NO_3^- decreased from the superficial sediments downwards, becoming depleted at about 3 cm depth in most sediment types and experimental conditions. There were no obvious changes in porewater nitrate profiles between treatments in the different sediment types, with the exception of the sediments from the unvegetated seagrass patch (higher values in both treatments, and depletion only at 10 cm in the treated sample) (Fig. 2).

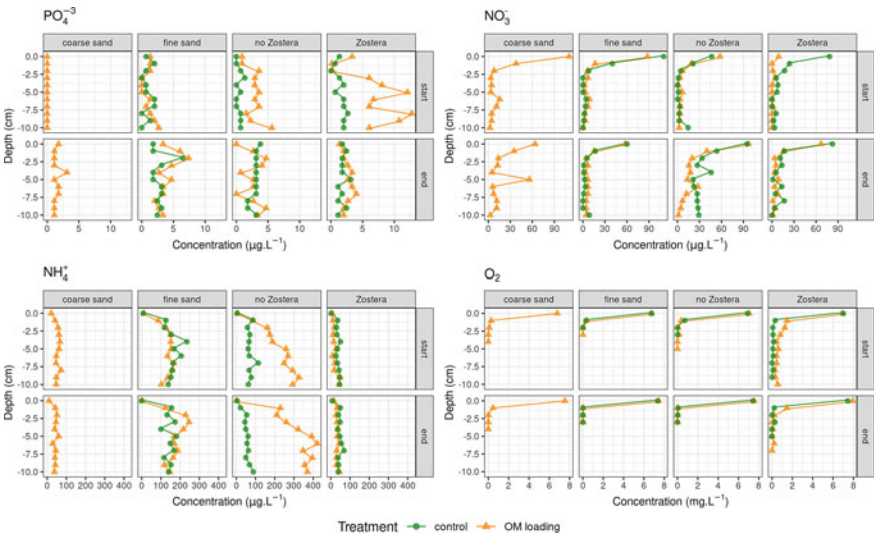

Fig. 2 Porewater nutrient and oxygen profiles in the different sedimentary habitat types under control conditions (green circles) and organic matter loading (orange triangles) at the start and the end of the experiment. 0 cm indicates the water immediately overlying the sediments

The porewater PO_4^{-3} concentrations were low, and increased with depth. By the end of the experiment, phosphate concentrations in pore waters had increased slightly, especially in the fine and coarse sand, and to a certain extent in the unvegetated seagrass sediment, in both control and treated samples. In the seagrass sediments, there was no change in porewater phosphates in the control, and an overall decrease under organic loading (Fig. 2).

3.2 Sediment-Water Interface Fluxes

All sediment types acted as a source of NH_4^+ throughout the experiment, and the magnitude of the flux increased under organic loading—sometimes as much as tenfold, e.g. in the fine sand (Table 3). By contrast, all were a sink of NO_3^-, and in most cases its uptake also increased under organic loading (with the exception of the sediments from the unvegetated patch in the seagrass bed). The PO_4^{-3} fluxes were

Table 3 Diffusive fluxes at the sediment-water interface of oxygen (O_2), ammonium (NH_4), nitrate (NO_3), and phosphate (PO_4) in different sediment types from Sozopol Bay at the start of the experiment and 39 days after extreme organic loading

Habitat type	Solute	Start		End	
		Control	Organic loading	Control	Organic loading
Coarse sand	O_2	–	13.22	–	50.42
	NH_4	–	−34.82	–	−208.13
	NO_3	–	126.16	–	180.62
	PO_4	–	0.00	–	1.33
Fine sand	O_2	12.12	12.03	28.17	91.12
	NH_4	−197.71	−125.54	−535.98	−1357.14
	NO_3	113.51	115.31	145.61	453.99
	PO_4	−0.70	0.00	0.00	−9.18
Zostera	O_2	35.18	28.66	30.54	40.99
	NH_4	−161.48	−20.97	−161.17	−100.00
	NO_3	246.18	40.21	243.11	283.45
	PO_4	0.98	4.69	−0.79	−1.18
No Zostera	O_2	20.57	22.23	20.72	14.32
	NH_4	−239.90	−235.93	−44.51	−392.96
	NO_3	73.06	111.18	101.48	94.62
	PO_4	0.00	0.00	0.53	−1.43

Fluxes are in mmol m^{-2} d^{-1} for the nutrients, and in mg m^{-2} d^{-1} for the oxygen. Positive values indicate solute uptake by the sediments, and negative—release to the water column. There are no values for control conditions in coarse sediments, because the sample was disturbed during transport and handling the smallest in magnitude; three of the sediment types subjected to organic loading had started releasing them to the overlying water at the end of the experiment, and only the coarse sands were taking them up.

4 Discussion

The exchange of nutrients between sediments and water is a complex phenomenon which depends on multiple physical, chemical and biological factors, such as temperature, dissolved oxygen, redox potential, benthic organism activities and organic matter.

Our results are comparable to other experimental studies that found that benthic nutrient regeneration generally increases with organic loading [24]. The high fluxes of ammonium and nitrates observed in our experiment reflect the strong early diagenesis of organic matter in all sediment types. This is also supported by the increased consumption of O_2 by the sediments in the organic loading treatment at the end of the experiment—especially high in the fine sands, which also exhibited the largest differences in nitrogen fluxes under organic loading. Spontaneous chemical oxidation of sulphide ions, released during sulphate reduction within the sediments, likely also contributes to the O_2 consumption by the sediments. Porewater NH_4^+ is probably mainly derived from the bacterial mineralization of the organic matter, which is supported by its general increase in concentrations under organic loading. The observed release of ammonium by the sediments and consumption of nitrates could be related to stimulated reduction of nitrates to ammonium within, in anoxic conditions, since by the end of the experiment, most sediment types had become anoxic below 2 cm depth—also consistent with the general increase of ammonium concentrations with depth in nearly all sediment types, and the parallel depletion of nitrates. In our experiment, NH_4^+ dominated the exchange of nitrogen between the sediment and the water column, and was the main form of nitrogen in sediment pore waters, which has also been observed in other similar studies [10, 25]. The increased consumption of nitrates by the sediments, as well as their depletion below 3 cm depth in all sediment types except the unvegetated seagrass patch, could indicate that denitrification is occurring at these depths [10], although [26] observed decreased denitrification rates under high organic loading.

The greatest differences in nutrient fluxes induced by the organic loading were observed in the fine sands, although all sediment types exhibited them. There were also differences in solute fluxes between the start and the end of the experiment in the controls.

The PO_4^{-3} porewater concentrations and fluxes in all sediment types and conditions during our experiment were much smaller than those observed in Varna Lake during the summer, where significant stratification and bottom hypoxia induce a P release from the sediments [15]. No hypoxic conditions were observed in the water layer overlying the sediments during our experiment, but by the end most sediments had become reduced at depths below 2 cm, and a release of PO_4^{-3} to the water column was present. Higher nitrate concentrations in the overlying water have been linked to slower phosphate release from reduced sediments [9], which could explain the smaller P fluxes observed.

The effects observed in our experiment could in part be due to the characteristics of the organic matter used for the loading. The amount and quality of organic matter reaching the sediments are known to influence the spatial variability and seasonal differences in sediment nutrient fluxes [27]. The algal organic matter used in this experiment is relatively refractory; an eutrophication-induced bloom is more likely to result in accumulation of phytoplankton-derived organic matter, which is more easily decomposed. The seagrass sediments are also naturally enriched in organic matter from the decomposition of seagrass biomass and epiphytes.

This study made apparent the high variability typical for coastal sediments, even at very small scales, which makes the interpretation of the results more difficult. The fluxes at the start of the experiment, before any treatment was applied, sometimes differed greatly between replicates of the same sediment type (e.g. Zostera). More replication should be considered in future studies to account for that.

The two-point study design also could be improved by including more intermediary measurements, which would allow to trace the immediate effects of the organic loading on the different sedimentary habitats.

Additionally, benthic faunal activities and behaviour may strongly influence nutrient cycling and fluxes by redistributing organic material, modifying sediment redox conditions, and creating chemical gradients and interfaces for solute exchange [28]. There are no studies on the effects of bioturbation on solute concentrations and fluxes in the Bulgarian Black Sea, and extrapolation from other seas is unlikely to be meaningful, since these processes are highly context-dependent even on a very local scale, and could be influenced by even small variations in environmental conditions [29]. Although this study did not take into account the macrofaunal communities in the different habitats, other studies have found that bioturbation and bioirrigation can alter near-surface porewater nutrient concentrations toward bottom water values [30]. Indeed, the ammonium and phosphate porewater profiles in our experiment are very similar to those in the sandy stations with high bioturbation potential observed by Gogina et al. [30], suggesting a possible mixing and transport effect.

Finally, our experiment only considered diffusive fluxes across the sediment-water interface. In natural conditions, and especially for permeable sandy sediments, advection (e.g. through sediment resuspension) can also be an important mechanism, sometimes exceeding diffusive fluxes, and should therefore also be taken into account [6, 31]. Our study area is probably advection-dominated—shallow, characterized by coarser sandy sediments, with potentially high physical exchange between bottom and interstitial water—and the solute fluxes are likely driven primarily by hydrological processes, with diffusion and bioturbation/biodeposition playing a secondary role [32].

Dense benthic macrophyte cover such as seagrass beds could also alter the porewater nutrient distribution and fluxes by capturing nutrients from the water column or from the sediments, and by increasing oxygen penetration in the sediments through their root systems [33]. Our results support this conclusion—oxygen penetration was highest in the seagrass sediments, there was generally little influence of organic loading on the nutrient profiles, and the sediment-water interface fluxes were more stable than in the other sediment types. This suggests that seagrass beds likely play a significant role in coastal water nutrient balance and water quality regulation.

5 Conclusions

Nutrient cycling in coastal marine sediments represents important ecosystem functions such as nutrient regeneration for primary producers and regulation of the ability to remove excess (natural or anthropogenic) nitrogen and phosphorus, which confer resilience to coastal zones through mitigating eutrophication. Although limited in scope, this study adds to the current knowledge of the influence of sediment type on the nutrient cycling and balance in the coastal Bulgarian Black Sea, and contributes towards our understanding of the role of coastal benthic habitats in water quality regulation. Future experiments will attempt to quantify the in situ fluxes in the main habitat types in Sozopol Bay, and compare them with previous in situ measurements from an impacted period before the construction of a wastewater treatment plant in the area. The experiments will also take into account the resident macrofaunal communities and their activities. This will allow a more realistic characterization of the sediment-water interface fluxes, and the effects of the interactions of multiple processes in coastal Black Sea habitats. Such results are especially valuable given the high context dependency and natural variability of these processes, and the consequent difficulty of extrapolating between regions.

Acknowledgements This study was financed by the Bulgarian National Scientific Program "Environmental Protection and Reduction of the Risk of Adverse Events and Natural Disasters" Approved by Council of Ministers Decision No 577/17.08.2018 and funded by the Ministry of Education and Science (Agreement No D01-230/06-12-2018), WP1.4.

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