Short-term effect of oxic to anoxic transition on benthic microbial activity and solute fluxes in organic-rich phytotreatment ponds

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Abstract Manipulative experiments to test the short-term effect of oxygen depletion events on microbial activity and benthic fluxes in organic-rich sediments were carried out in March and June 2004. Oxic–anoxic transitions were induced by prolonged dark incubation of sealed sediment cores collected in phytotreatment ponds. Benthic fluxes of oxygen (O_2), carbon dioxide (CO_2), inorganic nutrients, and free sulfides were measured before (oxic) and after (anoxic) the transition occurred. A multifactorial design was employed for monitoring esoenzymatic activity, heterotrophic bacterial production, total prokaryotic abundance, actively respiring bacterial cells, and the biochemical composition of sedimentary organic matter. The oxic to anoxic transition resulted in a significant increase of esoenzymatic activity and bacterial production in March, due to the profound modification of the benthic community and the release of labile organic compounds which followed the onset of anoxia. In parallel, net efflux rates of dissolved inorganic carbon (DIC) and ammonium (NH_4^+) sharply decreased, soluble reactive phosphorus (SRP) influx reversed, and sulfide was buffered within the oxidized sediments. From March to June, ponds evolved toward oxygen deficit and reducing conditions in the upper sediment horizon, losing benthic fauna and biogeochemical buffering capacity. Thus, the oxic to anoxic transition had a much smaller effect on microbial activity and net flux exchange, while S^{2-} was consistently delivered from the sediment to the water column. Overall data from this study suggest that the short-term response of benthic microbial activity and solute fluxes to anoxic events may have a significant impact on sediment biogeochemistry (e.g., at the oxic–anoxic interface), and that this impact may vary greatly depending on the sediment features, mainly its organic content and redox condition.

Keywords Oxic–anoxic transition · Microbial activity · Solute fluxes ·

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Organic-rich sediments · Biogeochemical buffers · Eutrophication

Introduction

In many brackish and shallow marine areas, increasing impacts of human activities arising from eutrophication, tourism, and aquaculture have resulted in dramatic changes in the structure and functioning of the benthic systems (Castel et al., 1996; Crossland et al., 2005; Rabalais et al., 2007). Demonstrated examples of such changes include the shift of primary producer communities from rooted phanerogams to drifting macroalgae, different abundance and community structures of meio- and macrofauna, decoupling between sedimentation and regeneration processes, anoxia, and occurrence of dystrophic events (Gee et al., 1985; Duarte, 1995; Schramm, 1999; De Wit et al., 2001).

Notwithstanding the great progress in studying processes that have major ecological consequences during the development of marine eutrophication (Kristensen et al., 1995; Jørgensen, 1996), crucial steps in the ecosystem shift from hypoxia to full anoxia are still questioned (Meyer-Reil & Köster, 2000). The unpredictable and non-linear behavior of stressed coastal ecosystems requires accurate measurements and powerful modeling tools to understand the key microbial and biogeochemical processes (Meyer-Reil & Köster, 2000). With this aim, several studies have been carried out to investigate benthic metabolism under the different environmental conditions which are commonly found before and after eutrophication is established. For example, the quali-quantitative nature of the mineralization processes within oxic and anoxic environments has been investigated, leading to a general conclusion that microbial biomass production and organic matter mineralization display lower rates under anoxic conditions, in particular, when the organic carbon bulk is mainly refractory (Kristensen et al., 1995; Sun et al., 1997; Amtoft Neubauer et al., 2004; Lomstein et al., 2006). The occurrence of steep vertical oxygen gradients and the establishment of an oxic–anoxic interface (OAI) within the sediment layers constitute a natural model for investigation of the oxic to anoxic transition (Brune et al., 2000). Studies on the structure and activity of the benthic community at the OAI have been carried out in sediment and microbial mats, where they highlighted a complex structural and functional organization of microbial groups in relation to a narrow stratification of physicochemical variables (Canfield & DesMarais, 1993; Bernard & Fenchel, 1995). Investigations applying molecular techniques have confirmed the occurrence of a distinct stratification of microbial populations in the vicinity of OAI and the identification of this area as the basis of key biogeochemical processes such as the aerobic terminal oxidation of reduced compounds by lithotrophic bacteria (Fenchel et al., 1998).

However, a full understanding of the ecological role which the OAI plays in marine sediment remains challenging. Holmer (1999) investigated the dynamic nature of the OAI, assessing the effect of oxygen depletion on organic matter mineralization in little to non-bioturbated marine sediments. Contrasting patterns were evidenced during the oxic to anoxic transition, with high mineralization rates recorded within the first few hours. The short-term response of the microbial activity following an oxygen depletion event is likely to play an important role in sediment biogeochemistry due to the dynamic nature of the OAI at micrometer scales. In fact, the oscillation of the OAI within the sediment matrix is primarily influenced by the sedimentation rates and the organic input. It is also dependent on diffusion processes, bioturbation activities, and the presence of rizophytes. Complex redox mosaics are the rule in marine sediments (Aller, 1994), and it is reasonable to assume that repetitive oscillations of the OAI make short-term responses of microbial activity quantitatively significant to the overall sediment biogeochemistry. In this context, organic enrichment of the coastal environment may profoundly affect OAI establishment and dynamics, thus affecting benthic metabolism and biogeochemistry (Amtoft Neubauer et al., 2004; Lomstein et al., 2006).

This study investigates the short-term effects of oxygen depletion on benthic microbial activity and biogeochemistry using a manipulative experimental approach. In particular, the authors present results from two experiments in which microbial activity and solute fluxes across the sediment–water interface were measured following induced anoxia in eutrophic brackish ponds receiving effluents from a marine fish farm.

Materials and methods

Study area

Surface sediments were collected from phytotreatment ponds receiving effluents from a land-based fish farm, which is located close to the Orbetello lagoon (Tyrrhenian Sea, central Italy) (Fig. 1). The phytotreatment plant consists of four ponds arranged in series with a surface area of $\sim$2,600 m^2 each and a mean depth of $\sim$0.7 m. Total water volume is 9,000 m^3 and 7,000 m^3 in the fish and phytotreatment ponds, respectively, and water flow is 140 l s^{-1}. Detailed descriptions of the fish farm, phytotreatment ponds, and main ecological studies were reported by Porrello et al. (2003) and Bartoli et al. (2005).

Owing to the limited hydrodynamic regime, water shallowness, and high organic load, phytotreatment ponds feature environmental conditions commonly found in coastal eutrophic environments, such as highly productive marginal areas of Mediterranean coastal lagoons that are subjected to summer collapse of primary production and anoxia (Castel et al., 1996; Viaroli et al., 1996; De Wit et al., 2001).

Water and sediment sampling, core maintenance, and incubation

Two intensive field campaigns were conducted in March and June 2004; on each occasion, water and sediment samples were collected. Water was sampled at the inlet and outlet of the phytotreatment plant according to the procedure described by Porrello et al. (2003) and filtered for the determination of dissolved and particulate nutrients.

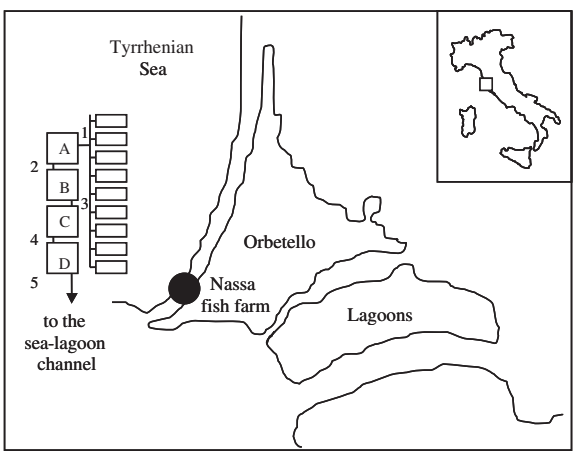

Fig. 1 Location of the study area and schematic representation of the fish farm and phytotreatment ponds. *Squares* represent phytotreatment ponds A–D; *numbers* indicate inlet and outlet sampling sites for water. Sediments were collected approximately at the center of each pond

Intact sediment cores (transparent plexiglass liners, 8 cm i.d., length 30 cm) were collected manually at each pond. Sediment was leveled to a 12-cm height, and the water column overlying sediments was approximately 14 cm. On each sampling date, six cores were collected from each pond for a total of 24 sediment cores. Immediately after collection, the cores were submerged in tanks containing in situ aerated water and were left overnight to allow for sediment stabilization. Sediment–water fluxes of O_2, dissolved inorganic carbon (DIC), NH_4^+, soluble reactive phosphorus (SRP), and S^{2-} were measured in 12 cores via dark incubations starting from the morning after the sampling. The remaining cores were left submerged in oxic water for later surface sediment characterization.

Incubated cores were sealed with floating lids; water stirring was ensured by Teflon-coated magnetic bars rotating at 40 rpm. A detailed description of core pre-incubation and incubation techniques employed in this study is reported in Dalsgaard et al. (2000). Compared to standard flux measurements, the dark incubation was prolonged up to the establishment of anoxic conditions in the water column. The incubation time was set on the basis of oxygen measurements to entirely deplete the oxygen reserve in the water column and was protracted under anoxic conditions for a maximum of $\sim$20 h. Following this rationale, the March incubation lasted 44 h, while the June incubation lasted 25 h. Water samples were collected with different timing under oxic and anoxic conditions for a total of eight samplings. Following each collection, sampled water (representing less than 10% of the water volume inside the core) was replaced with water from the incubation tank. Rates for the oxic part were estimated generally within the initial 2 h, while rates in the anoxic part were calculated after a few hours (2–3) from the disappearance of dissolved oxygen in the water column of each core.

Sediment characterization

The upper sediment horizon (0–2 cm depth) of the cores maintained in oxic conditions was sliced, mixed

thoroughly, and analyzed for the determination of sediment features and microbial activity. In addition, microbial activity and organic matter quality were measured in the incubated cores at the end of the incubations.

Total organic carbon (TOC) and total nitrogen (TN) were determined with a CHNS-O EA 1108 Elemental Analyzer (Carlo Erba); total phosphorus (TP) was determined spectrophotometrically after acid extraction from ashes (Aspila et al., 1976). Proteins (PRT) were determined according to Hartree (1972); carbohydrates (CHO) were analyzed according to Dubois et al. (1956).

The total labile iron pool (LFe, defined as LFe(III) + LFe(II)), acid volatile sulfide (AVS, which includes S^{2-} and FeS), and the chromium-reducible sulfur (CRS, which is mainly composed of FeS_2 and S^0) were analyzed with methods reported by Azzoni et al. (2005).

Enzymatic activity

Analyses of aminopeptidase (L-Leucine-4-methylcoumarinyl-7-amide, Leu-MCA) and β-glucosidase (MUF-β-glucopyranoside, Glu-MUF) enzymatic activity were carried out by adding 1 ml of Leu-MCA and Glu-MUF, respectively (final concentration 200 μM), as described by Hoppe (1993). Undisturbed sediment sub-samples (0.5 ml) were incubated for 1 h in the dark and at in situ temperature and then centrifuged (3,000 rpm, 1,000×g, 5 min). Enzymatic rates were assessed by fluorometric analysis at 380 nm excitation, 440 nm emission (Meyer-Reil, 1986) for MCA, and at 365 nm excitation; 455 nm emission (Hoppe, 1993) for MUF. Data were normalized to dry weight after desiccation (60°C, 24 h) and reported as nanomoles of Leu-MCA or Glu-MUF released per gram of sediment dry weight h^{-1}. Esterase activity was assayed with fluorescein diacetate (FDA, Sigma, St. Louis, USA) according to Battin (1997). Samples were incubated at a final concentration of 200 μM FDA. After a 30-min incubation period, the hydrolysis was stopped with acetone, and the samples were stored in ice. Fluorescein was extracted by sonicating (45 s, 30 W output) the suspension, and particles were removed by centrifugation (8°C, 20 min, 5,000 rpm). The absorbance of the supernatant was measured spectrophotometrically at 490 nm. Enzymatic activity was converted into equivalents of C mobilized assuming that 1 nmol of substrate hydrolyzed corresponded to 72 ng of mobilized C (Manini et al., 2003).

Heterotrophic bacterial production

Heterotrophic benthic bacterial production (HBP) was measured using ^{3}H-leucine incorporation (Van Duyl & Kop, 1994). Sediment sub-samples (200 μl) added to 26 μl of ^{3}H-leucine (6 μCi final concentration per sample) were incubated for 1 h in the dark at in situ temperature. After incubation, bacterial C incorporation was stopped with 1.7 ml of 80% v/v ethanol before scintillation counting. Sediment blanks were made by adding ethanol immediately before ^{3}H-leucine addition. Data were normalized to sediment dry weight after desiccation (60°C, 24 h).

Total prokaryotic abundance (TPA) and actively respiring cells (ARC)

For a total direct count of prokaryote cells, sediment samples were sonicated three times (Sonifier Labor 2000, 195 W for 1 min). Sub-samples were diluted 250 times. The number of prokaryote cells in 0.2-μm black Nucleopore filters was estimated using the Acridine Orange Direct Count technique (Hobbie et al., 1977) with epifluorescence microscopy (Zeiss Universal Microscope). Actively respiring cells in the sediment were determined following Proctor & Souza (2001). In brief, 1.3-mm depth of seawater was gently pipetted onto homogenized sediment slurries (0.5 ml). Under red light, 250 μl of the fluorogenic redox dye 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) working stock was injected into the 2-cm sediment slurries with a 26-G needle, lifting the needle upward from the bottom through the sediment section. The final concentration of CTC was 5.5 mM (Bhupathiraju et al., 1999). The assay was incubated in the dark for 4 h at room temperature, and the reaction was stopped with 3 ml of 20% formalin and stored at −20°C. An aliquot of the supernatant was filtered through a 0.2-mm pore-size black Nuclepore membrane filter. Then, microscope slides were prepared, and the numbers of actively respiring bacteria were counted by epifluorescence microscopy (Zeiss Universal Microscope).

Analyses of solutes and particulate carbon, nitrogen, and phosphorus

Dissolved oxygen was determined with the Winkler method (APHA, 1975) and with polarographic Clark-style microelectrodes (Unisense, DK). Total dissolved inorganic carbon was measured by titration with 0.1 N HCl (Talling, 1973). Ammonium (Bower & Holm Hansen, 1986), SRP (Valderrama, 1977), and sulfide (Cline, 1969) were determined spectrophotometrically. Water samples were filtered on GF/F Whatman filters for particulate organic carbon (POC) and particulate nitrogen (PN) and processed with a CHNS-O EA 1108 Elemental Analyzer (Carlo Erba). Particulate phosphorus (PP) was determined as soluble reactive phosphorus following persulfate oxidation and extraction from filters (Valderrama, 1981).

Statistical analyses

A two-way ANOVA was used to test the null hypothesis that solute flux values and microbial activity rates were the same in the oxic and anoxic cores following the induced oxic–anoxic transition. Factors in the model were "Transition [T]" (two levels, orthogonal, fixed) and "Pond [P]" (four levels, orthogonal, fixed). Three replicates were employed in the analysis. The effect of the oxic to anoxic transition was then investigated by testing whether the main factor [T] was statistically significant ($P < 0.05$). In order to interpret statistically significant interactions among the different treatments, a SNK post-hoc test was carried out. Prior to the analysis, the homogeneity of variance was tested by Cochran's test and, when necessary, data were appropriately transformed. Pearson correlation analysis was carried out to test relationships among microbial and environmental variables. All statistical tests and correlation analyses were performed using the MATLAB Statistics Toolbox (Version 6.1; TheMathWorks).

Results

General features of effluents and surface sediments

Chemical analyses on effluents from the fish farms indicated an increase of nitrogen species concentrations from March to June (Table 1). The increase in suspended solids and development of algal mats within the phytotreatment ponds also resulted in higher retention of particulate matter within the four basins (Table 1).

Table 1 Physicochemical features of the fish-farm effluents entering into phytotreatment pond A, and net daily balances of particulate carbon, nitrogen, and phosphorus in March and June 2004. For the latter, a negative balance indicates a retention of particulate matter within the phytotreatment ponds (= sedimentation)

Variables	March	June
T (°C)	14.6 ± 0.8	22.6 ± 0.9
O_2 (%)	75.7 ± 11.4	66.3 ± 4.7
NH_4^+ (μM)	37.7 ± 5.1	61.4 ± 4.6
SRP (μM)	3.2 ± 0.5	2.8 ± 0.5
TDN (μM)	64.7 ± 5.6	113.1 ± 30.4
TDP (μM)	3.4 ± 0.6	3.1 ± 0.4
POC (μM)	61 ± 4	95.8 ± 11.8
PON (μM)	8 ± 0.2	14.8 ± 1.33
PP (μM)	0.7 ± 0.2	1.2 ± 0.3
C-sed (mol d^{-1})	−367	−1,095
N-sed (mol d^{-1})	−46	−163
P-sed (mol d^{-1})	−2.7	−13

T temperature, O_2 oxygen, NH_4^+ ammonium, *SRP* soluble reactive phosphorus, *TDN* total dissolved nitrogen, *TDP* total dissolved phosphorus, *POC* particulate organic carbon, *PON* particulate organic nitrogen, *PP* particulate phosphorus, *C-sed* particulate carbon, *N-sed* particulate nitrogen, *P-sed* particulate phosphorus

Sediments collected in March were light brown in the upper horizon and bioturbated; benthic macrofauna displayed increasing abundances from pond A to pond D. The dominant species were represented by the Polychaeta *Neanthes caudata* (up to 19,500 ind m^{-2}, pond B), *Malacoceros fuliginosus* (up to 5,000 ind m^{-2}, ponds C and D), the Crustacea *Microdeutopus gryllotalpa* (up to 6,000 ind m^{-2}, pond B), and *Corophium insidiosum* (up to 12,000 ind m^{-2}, ponds B and C). In June, surface sediments were dark brown and defaunated (Tomassetti, pers. comm. Central Institute for the Scientific and Technologic Research applied to the Sea), with the exception of *Neanthes caudata* (up to 3,100 ind m^{-2}, pond B).

Total carbon, nitrogen, and phosphorus in surface sediments were similar in the two sampling periods; while proteins increased markedly from March to

Table 2 Main features of surface sediment (upper 2 cm) collected from the four phytotreatment ponds in March and June 2004

Pond	Date	TOC (%)	TN (%)	TP (%)	PRT (mg g^{-1})	CHO (mg g^{-1})	LFe (μmol cm^{-3})	AVS (μmol cm^{-3})	CRS (μmol cm^{-3})
A	March	4.9 ± 1.3	0.8 ± 0.1	0.2 ± 0.0	0.56 ± 0.19	1.12 ± 0.56	16.2 ± 1.3	5.3 ± 2.8	57.7 ± 5.0
	June	4.9 ± 0.6	0.9 ± 0.1	0.2 ± 0.1	5.60 ± 0.27	6.14 ± 4.62	21.7 ± 6.5	26.7 ± 2.2	23.8 ± 3.4
B	March	4.7 ± 0.8	0.7 ± 0.1	0.2 ± 0.0	2.06 ± 0.40	5.84 ± 1.42	20.2 ± 3.7	13.2 ± 6.4	61.9 ± 11.8
	June	6.2 ± 0.4	0.9 ± 0.1	0.4 ± 0.1	8.58 ± 0.74	10.56 ± 4.42	59.5 ± 3.5	27.5 ± 1.8	57.4 ± 8.2
C	March	5.3 ± 0.5	0.7 ± 0.1	0.3 ± 0.0	0.31 ± 0.64	8.37 ± 1.24	34.6 ± 3.9	14.7 ± 3.4	105.1 ± 9.0
	June	3.8 ± 0.6	0.5 ± 0.1	0.3 ± 0.1	7.46 ± 1.51	7.73 ± 4.04	44.6 ± 9.5	25.5 ± 3.1	100.8 ± 13.0
D	March	4.3 ± 0.3	0.6 ± 0.0	0.2 ± 0.1	2.18 ± 0.73	7.04 ± 1.47	34.0 ± 3.2	22.0 ± 1.7	75.7 ± 14.5
	June	5.7 ± 0.6	0.9 ± 0.1	0.3 ± 0.0	6.99 ± 0.40	10.47 ± 7.00	52.4 ± 5.7	22.9 ± 4.0	85.0 ± 7.1

TOC total organic carbon, *TN* total nitrogen, *TP* total phosphorus, *PRT* proteins, *CHO* carbohydrates, *LFe* total labile iron, *AVS* acid volatile sulfide, *CRS* chromium-reducible sulfur

June (Table 2); the carbohydrate increase was significant only in ponds A and B. From March to June, total labile iron, which represents the more reactive iron pool, increased in all ponds. In parallel, a significant increase in AVS production was observed mainly in the first basins which receive the greater organic loads. CRS exhibited significant changes only in pond A where it decreased from 57.7 to 23.8 μmol S cm^{-3}, while in the other three ponds, it did not undergo significant changes. The total reduced sulfur (TRS = AVS + CRS) remained almost constant from March to June, while an increase was observed from ponds A and B to ponds C and D, thus evidencing a better capacity of accumulating reduced sulfur in the final ponds.

Short-term effect of oxic to anoxic transition on the microbial activity

The oxic–anoxic transition induced a significant increase in esoenzymatic and microbial activities in March 2004, especially for esterase (FDA), which also increased from pond A to pond D (Fig. 2). Heterotrophic bacterial production displayed an even larger increase in all cores and shifted on average from 1,927 ± 432 ng C g^{-1} h^{-1} (oxic) to 3,566 ± 427 ng C g^{-1} h^{-1} (anoxic) (ANOVA, $p < 0.05$). A significant change in the composition of sedimentary organic matter was also observed following the transition, with an increase in carbohydrate and a decrease in the protein contributions to total biopolymeric carbon. No significant changes were observed in the total prokaryotic density, with 3.6 ± 1.9 cells × 10^9 g^{-1} under oxic conditions and 3.5 ± 1.3 cells × 10^9 g^{-1} under anoxia (ANOVA, $p > 0.05$). The actively respiring cells were slightly higher in the anoxic (average 37 ± 3%) than in oxic (average 31 ± 3%) cores.

In June, the microbial activity increase following the transition was not further observed (Fig. 2). The aminopeptidase activity displayed higher rates in the oxic cores (ANOVA, $p < 0.05$). No significant changes were observed for glucosidase and esterase rates, or for heterotrophic bacterial production (ANOVA, $p > 0.05$). Furthermore, the total prokaryotic density (19.0 ± 7.9 cells × 10^9 g^{-1} oxic and 17.3 ± 9.5 cells × 10^9 g^{-1} anoxic) and the number of actively respiring cells (32.0 ± 5.3% oxic and 31.0 ± 4.8% anoxic) displayed similar values in both oxic and anoxic cores.

Short-term effect of oxic to anoxic transition on the solute fluxes

An example of the evolution of solute concentrations in the water overlying the sediments in the cores collected at pond A during the oxic–anoxic transition is given in Fig. 3. During the oxic part of the incubation, oxygen consumption was coupled to a net regeneration of inorganic carbon from pond sediments to the water column, while only in June, a great sulfide efflux was detected just after the onset of anoxia.

Rates of solute net exchange across the sediment–water interface calculated in the oxic and anoxic parts of the incubation changed following different patterns among ponds and seasons.

Sediment oxygen demand (SOD) was significantly higher in pond A in March and June (−6.06 ± 0.17

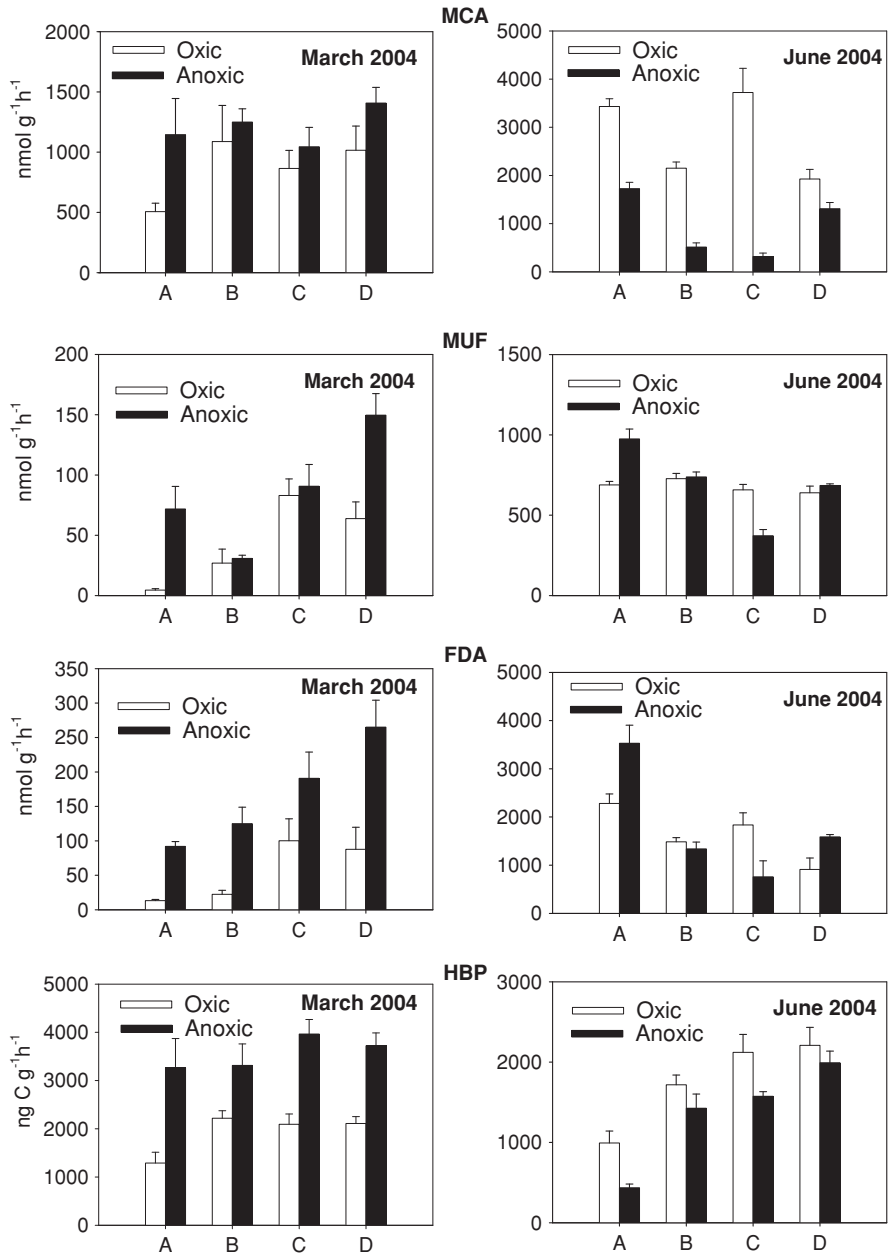

Fig. 2 Rates of esoenzymatic activity and heterotrophic bacterial production in oxic and anoxic sediment cores following the experiment in March and June 2004. *MCA* aminopeptidase, *MUF* glucosidase, *FDA* esterase, *HBP* heterotrophic bacterial production

and -13.89 ± 1.03 mmol O_2 m^{-2} h^{-1}, respectively, ANOVA, $p < 0.01$); in ponds B, C, and D, rates were similar and ranged between -1.78 and -4.21 mmol O_2 m^{-2} h^{-1} (Fig. 4).

DIC efflux rates were significantly higher at pond A where they increased sharply from March (5.78 ± 1.48 mmol m^{-2} h^{-1}) to June (29.66 ± 4.19 mmol m^{-2} h^{-1}). Rates were not significantly different among ponds B, C, and D and the sampling dates (ANOVA, $p > 0.05$) even though there was a general tendency toward higher DIC effluxes in June. The respiratory quotient, calculated as the ratio between DIC and $|O_2|$ dark fluxes (Dilly, 2003) averaged 1.0 in March and 2.1 in June. The shift between oxic and anoxic conditions resulted in a significant decrease of DIC efflux (ANOVA, $p < 0.01$) in the March incubation; the relative decrease was minimum at pond A (-39%) and maximum at pond D

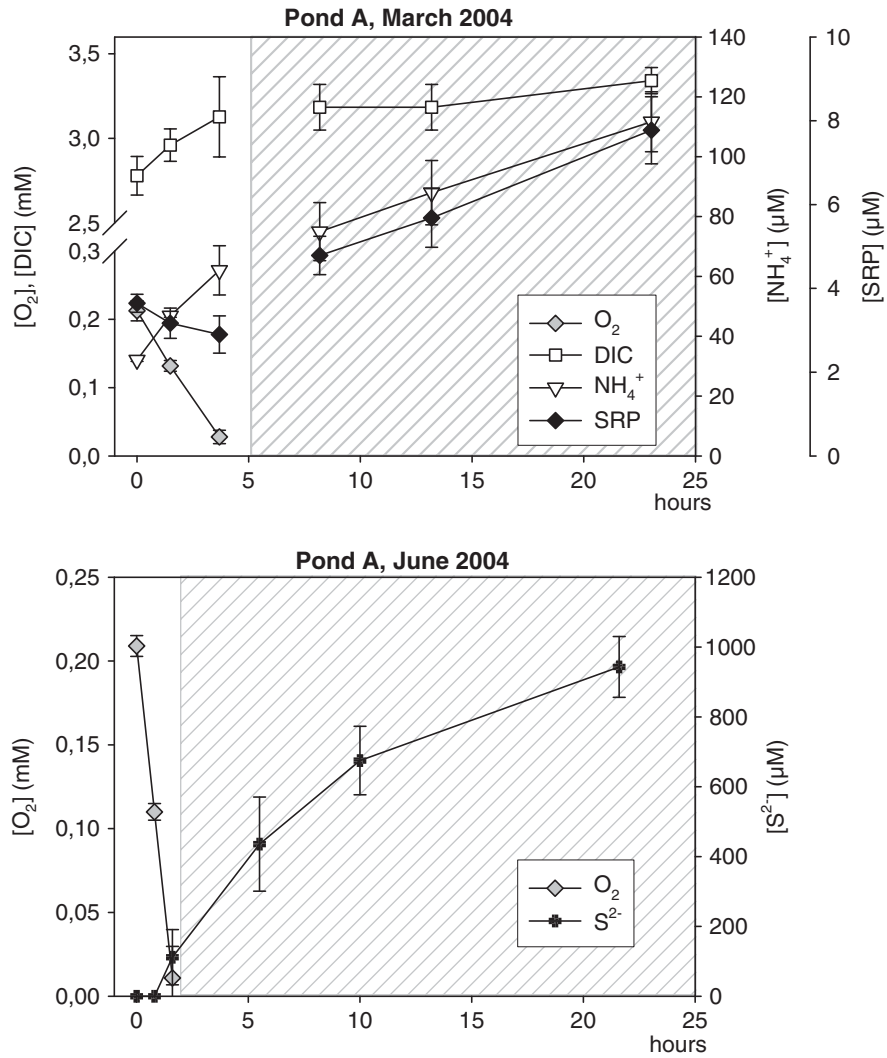

Fig. 3 Evolution of O_2, DIC, NH_4^+, SRP, and S^{2-} in the water column during oxic–anoxic transitions in sediment cores collected in pond A in March (*upper panel*) and in June (*lower panel*) 2004. The *hatched area* indicates the anoxic part of the incubation. The March incubation lasted 44 h, but sulfide efflux to the water column was not detected; the June incubation was shorter, as sulfides migrated in the water column also under hypoxic conditions

(−82%). Induced anoxia led to a general decrease in DIC efflux (−7 to −35%) also in June, even though differences with oxic incubations were not significant (ANOVA, $p > 0.05$).

Sediments from all ponds regenerated large amounts of ammonium on both sampling dates (Fig. 5). Efflux rates measured in the oxic part of the March incubation were found between 794 (pond D) and 1,202 µmol m^{-2} h^{-1} (pond A); differences between sampling sites were not significant. In June, the highest NH_4^+ aerobic flux was determined at pond A (4,176 ± 314 µmol N m^{-2} h^{-1}), while at ponds B, C, and D rates were similar to those determined in March and occurred between 442 and 1,080 µmol m^{-2} h^{-1}. As anoxia established, the net release of ammonium was significantly attenuated, with the exception of pond D, June incubation (Fig. 5). The relative decrease determined in March overlapped that just described for DIC with a minimum in pond A (−15%) and a maximum in pond D (−76%). In June, the picture was different, with a greater effect of induced anoxia in ponds A and B, where the anoxic flux was three times smaller than the corresponding oxic flux. At pond C, rates measured in anoxic conditions tended to be only somewhat smaller (−23%), while at pond D, rates were similar.

Surface sediments of ponds A, B, and C were a net sink for SRP during the aerobic phase of the March incubation, with fluxes varying between −48 and

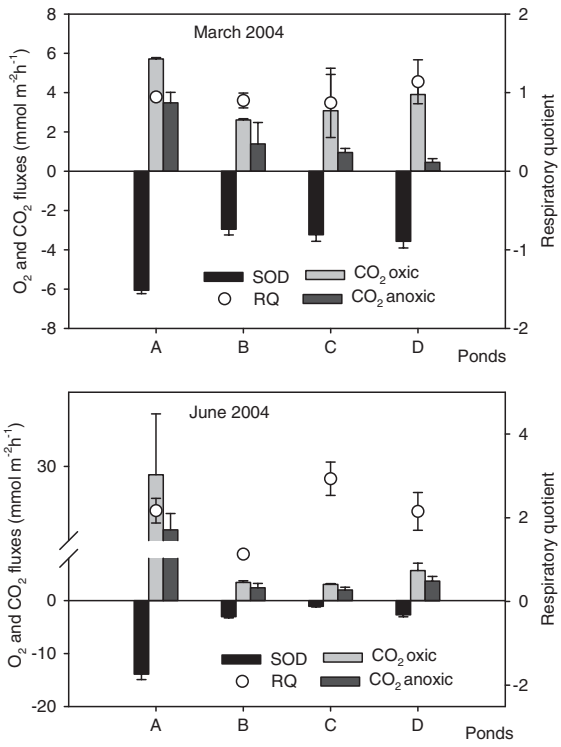

Fig. 4 Dark sediment O_2 demand (SOD), CO_2 net efflux (= DIC), and respiratory quotient ($RQ = CO_{2\ oxic}$ flux/|O_2 flux|) measured in March and June 2004 in the four phytotreatment ponds before and after the oxic–anoxic transition has occurred. Each bar is the average of three measurements; standard errors are reported

-8 μmol SRP m^{-2} h^{-1}, while SRP regeneration was measured at pond D (12 ± 4 μmol m^{-2} h^{-1}). This picture was similar during the oxic incubation of June, except for sediments from pond A that were a net source of inorganic phosphorus (273 ± 90 μmol m^{-2} h^{-1}) (Fig. 5). Overall, the oxic–anoxic transition reversed SRP influx or enhanced SRP efflux to the water column; a significant effect was determined in March (ANOVA, $p < 0.01$) but not in June. In pond D where SRP flux was directed to the water column during the oxic part of the incubation, regeneration rates increased by 50% and 36% in March and June, respectively, after the transition to anoxia. In June, in pond A, SRP efflux decreased significantly (-62%), shifting from 273 ± 91 to 105 ± 31 μmol m^{-2} h^{-1}.

Efflux of free sulfides in the water column was undetectable in March, even after more than 20 consecutive hours of anoxia. The situation was different in June, with marked differences among sites. In pond A, after just 1.6 h of incubation, the water column of the cores was anoxic and sulfides started migrating out of the sediments with a rate of $3{,}966 \pm 1{,}293$ μmol m^{-2} h^{-1} (Fig. 6); at ponds D and B, sulfides were detectable after 11 and 16 h, respectively, with rates of $1{,}747 \pm 501$ and $1{,}024 \pm 1{,}040$ μmol m^{-2} h^{-1}. At pond C, sulfide efflux was much lower, and only few micromoles were determined in the water column during the last sampling, after 21 h of incubation (Fig. 6).

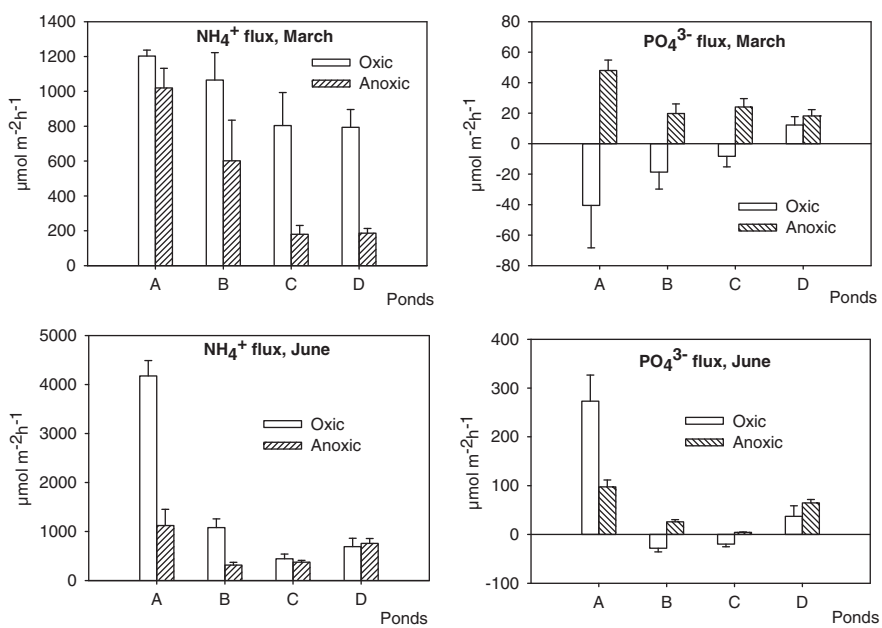

Fig. 5 Sediment–water fluxes of NH_4^+ and PO_4^{3-} measured in March and June 2004 in the four phytotreatment ponds during the oxic and the anoxic parts of the incubation. Each bar is the average of three measurements; standard errors are reported

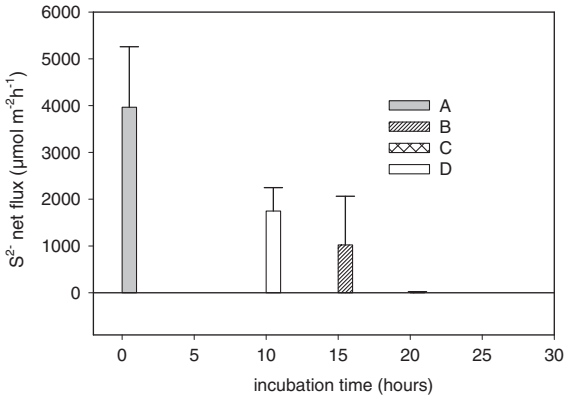

Fig. 6 Net sediment–water fluxes of free sulfides measured during the oxic–anoxic transition in the four fishponds. Rates were calculated from linear fitting of concentrations versus time, using the time at which S^{2-} was detectable in the water column at time zero

Discussion

Oxic to anoxic transition events are very common in aquatic sediments and may occur as sharp changes in environmental conditions within short spatial (mm) and temporal (h) scales. Although a number of studies have been carried out to investigate both the structure and activities of microbial populations at the oxic–anoxic interface and the long-term effects of anoxic events, little is known about the dynamic role of the OAI and the effect of short-term redox oscillations on sediment metabolism and flux rates across the interface. This is particularly true in benthic systems such as those investigated in this study that undergo large seasonal changes in sedimentary pools, the macromolecular quality of organic matter, and colonization by meio- and macrobenthos.

The phytotreatment ponds studied in this research are artificial systems that have been running for more than 10 years; their sediments host variable numbers of tolerant meio- and macrofauna species and are subject to different organic loads from winter to summer due to increased fish feed and fish biomass in the farm, which significantly alter benthic features and metabolism (Bartoli et al., 2005). Accordingly, the different effects of short-term oxic to anoxic transition on sediment metabolism and flux rates across the interface observed in the March and June incubations are a consequence of the change in sediment conditions which occurred between the two dates. If the total C, N, and P in surface sediments were comparable in the two sampling dates, the total amount of particulate matter settling on the ponds' sediment was about three times higher in June. This input of labile organic matter resulted in significantly higher concentrations of carbohydrates and proteins that fed microbial metabolism. In turn, enhanced metabolic rates induced the reduction of electron acceptors, with a parallel redox decrease and lower sedimentary buffering capacity.

The respiratory quotient (RQ), the ratio of dark CO_2 production to oxygen uptake rates, was used as a proxy for the degree of coupling reduction and re-oxidation processes. RQ should be close to 1 under balanced conditions, that is, when all organic matter is metabolized aerobically or any reduced products of anaerobic respiration are completely re-oxidized (Andersen & Kristensen, 1988). Values substantially greater than 1 indicate incomplete re-oxidation and accumulation in the sediment of the end products of anaerobic respiration. Thus, the shift from 1 to 2.1 from March through June could indicate that the system accumulated reducing compounds. This interpretation conforms to AVS data which clearly demonstrated that there was an accumulation of reduced compounds in the June sampling, especially in ponds A and B which received the fish-farm effluent. From March to June, the AVS to AVS + CRS ratio, which is considered an index of potential risk of dystrophy (Azzoni et al., 2005), increased especially in pond A (by a factor 6) and to a less degree in ponds B (2) and C (1.6). Organic matter loads to the sediment, coupled to the onset of anaerobic metabolism, weakens or depletes the sedimentary buffering capacity against sulfides, thereby increasing the system vulnerability to dystrophic events. In March, sediments were incubated anaerobically for about 20 h, and no free sulfides accumulated in the water column, meaning that they were efficiently retained within sediments by ferrous iron (Rozan et al., 2002; Rickard & Morse, 2005 and references therein). In June, after the exhaustion of geochemical buffers, anoxia was followed by an immediate release of S^{2-} from reducing sediments of pond A, and to a lesser extent and with a longer time lag, from ponds D, B, and C.

Reducing conditions at the benthic level were responsible for the collapse of macrofauna communities (Diaz & Rosenberg, 1995; Gamelink et al., 1996; Hyland et al., 2005). Bioturbation by macrofauna has

a well-demonstrated effect on flux rates across the sediment–water interface and enhances the bacterial activity through particle reworking and transport in deep sediments of electron acceptors (Aller, 1980; Kristensen, 1988; Pelegrì and Blackburn 1995; Nizzoli et al., 2007).

Experimental measurements following the induced oxic to anoxic transition within sediment cores displayed a significant increase of microbial enzymatic activity rates and bacterial production during the March experiment. A higher percentage of actively respiring cells was also observed in anoxic cores, indicating an increase of the active bacterial fraction notwithstanding the depletion of dissolved oxygen. These results are consistent with the findings of Holmer (1999) and Amtoft Neubauer et al. (2004) who reported a stimulation of sulfate reduction rates after the initial phase (6–48 h) of oxygen depletion events within marine sediments. Increased mineralization when an oxic condition changes to anoxic is also referred to as "nutrient flush" in soil studies, where the dynamic nature of OAI is known to stimulate decomposition of bacterial biomass caused by rapid environmental change (Aller, 1994).

Rapid environmental change due to redox oscillations may thus stimulate the release of labile organic substrates and promote microbial decomposition. The lowest protein-to-carbohydrate ratio observed in March after the transition reflected this event and suggested a rapid exploitation of the labile protein fraction by the microbial community. The breakdown of organic polymers by enzymatic attack contributes to the transformation of particulate organic carbon (POC) into dissolved organic carbon (DOC) which therefore becomes available for respiration (Unanue et al., 1999). In March 2004, the amount of DOC release was indirectly estimated assuming that the assimilation of 1 ng C g^{-1} h^{-1} corresponds to the processing of 4 ng C g^{-1} h^{-1} when Bacterial Growth Efficiency (BGE) is 0.2, with a BGE of 0.5 being the maximum in eutrophic waters (Del Giorgio & Cole, 1998). Assessing the total potential mobilized carbon (TPMC) from enzymatic activity measurements, we estimated that with a BGE of 0.2, $\sim$5% of TPMC was converted into bacterial biomass, $\sim$20% was respired, and the remaining $\sim$75% was released as DOC by incomplete degradation of organic polymers. These results are in agreement with Kristensen et al. (1995), Andersen (1996), Holmer & Kristensen (1996), and Holmer (1999), who demonstrated that under anoxic conditions, a lower CO_2 production was accompanied by an accumulation of dissolved organic carbon in pore waters.

Interestingly, the short-term increase in bacterial production following oxygen depletion was not coupled with an increase of the total mineralization rates; these, in contrast, underwent a rapid decrease as evidenced by DIC and NH_4^+ fluxes under anoxic conditions, which were about half of those measured under aerobic conditions. Such results could be explained in terms of less efficient or incomplete organic matter oxidation by the anaerobic microbial metabolism. Meio- and macrofauna can also exert an active role in the POC breakdown and are also responsible for a major fraction of solute exchange through the interface (Kristensen & Hansen, 1999; Kristensen & Holmer, 2001).

Oxygen depletion had an opposite effect on SRP dynamics, with phosphorus largely dependent on several biogeochemical factors, that is, ferric iron pool, carbonates, pore-water pH, and redox sulfur cycling (Heijs et al., 2000; Rozan et al., 2002). SRP influx during oxic incubation was the probable consequence of precipitation with ferric iron in bioturbated sediment, whereas SRP efflux under induced anoxia was a consequence of iron reduction and SRP mobilization.

The significant effect of the oxic–anoxic transition on microbial activity was not further observed during the June experiment. This was also true in terms of the percentage of actively respiring cells, which displayed similar values in both oxic and anoxic cores. Enzymatic activities showed higher rates at the start of the experiment in relation to the highest concentration of sedimentary organic matter, while bacterial production displayed lower values most probably linked to a low BGE (Del Giorgio & Cole, 1998). Nevertheless, delta values calculated as the arithmetic difference between anoxic and oxic rates in each experimental core for both enzymatic activity and bacterial production were lower when compared to those of the March experiment. Accordingly, the oxic–anoxic transition resulted in a lower reduction of NH_4^+ efflux and a not significantly different inorganic carbon production. It is likely that organic carbon oxidation in these sediments proceeds with comparable rates in aerobic and anaerobic conditions. This can be explained in terms of the lability of

settling particles (fish feces and not-ingested food together with fragments of macroalgae) and the nature of the microbial communities that are adapted to a reducing and sulfidic environment (Kristensen et al., 1995). In pond A, which received the highest load of particulate organic matter from the fish farm, DIC efflux was very high during oxic incubation (29.7 ± 4.2 mmol m^{-2} h^{-1}), but it decreased by only 7% during the transition. Excluding results from this pond which can be biased by high solute concentrations in the closed experimental system, oxic–anoxic transition had a significant effect on SRP fluxes with the anaerobic flux higher than the corresponding aerobic fluxes.

Conclusion

These results all fit with the general hypothesis that when sediments characterized by a relatively low organic matter concentration (as is the case during the March experiment) are exposed to anoxia, the aerobic community will be damaged resulting in the sudden release of labile DOC. Under these conditions, the anaerobic community will respond positively to the DOC input. In contrast, the oxic zone in heavily loaded sediments (as is the case during the June experiment) is much narrower, which implies that oxic–anoxic transitions will only have a minor impact on the sediment biogeochemistry.

The short-term increase in microbial activity rates following induced anoxia suggests that repetitive short-term oscillations in both OAI and oxic–anoxic conditions may be a critical factor not only affecting rates of microbial mineralization but also the biological decomposition of organic polymers. The DOC which is released from enzymatic processing may accumulate within the sediment or diffuse upward into the upper sediment layers and efflux into the water column. This latter sequence of processes has been shown to fuel pelagic microbial mineralization, representing an additional important process involved in the sediment metabolism (Fabiano et al., 2003; Gallizia et al., 2004). As a general statement, the authors speculate that the dynamic nature of OAI may play a significant role in sediment biogeochemistry by stimulating microbial activities over the short term. This role may vary based on different sediment conditions and can be strongly affected by organic matter loadings derived from direct sewage discharge and/or produced within the aquatic system as a consequence of eutrophication. Under changing conditions—for example, increased organic loads coupled with increased temperatures—the occurrence and frequency of oxic–anoxic transitions can be expected to saturate the sedimentary buffering capacity as well as to shift the benthic system toward azoic and rather unstable conditions. In other words, step-by-step the system would become more vulnerable to external stressors and less resilient until the shift would become irreversible.

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